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THE VACCINE TREATMENT OF ACUTE ANTERIOR POLIOMYELITIS OF MONKEYS*

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As previously reported by Kolmer, Klugh and Rule[1] it would appear that the subcutaneous injection of the Kolmer vaccine of 4 per cent suspensions of remote monkey passage virus attenuated by 1 per cent solutions of sodium ricinoleate are capable of rapidly producing antiviral antibody in both monkeys and human beings. Thus a monkey weighing 4 kg. inoculated intracerebrally under ether anesthesia with 0.5 cc. of 5 per cent virus 72 hours after a subcutaneous injection of 0.5 cc. of vaccine remained perfectly well and entirely free of infection, whereas a control developed paralysis 8 days after inoculation with but 0.1 cc. of the same virus given at the same time. Furthermore, the serums of three children were found to contain antiviral antibody when tested 4 days after the first subcutaneous injection of vaccine, the serums of these children being free of antibody before the vaccine was given.

Under the circumstances it would appear that the vaccine may be used during epidemics of acute anterior poliomyelitis and the rapidity of antibody production also suggests that it may be helpful during the incubation period of disease and possibly also when given during the early stage of an attack.

The purpose of this investigation was therefore to determine more accurately the protection value of the vaccine when given to monkeys at varying intervals during the incubation period of the disease following intracerebral inoculations of virus and also to determine if it had any demonstrable value in mitigating the

* Read before the Fourteenth Annual Convention of the American Society of Clinical Pathologists held at Atlantic City, June 7 to 9, 1935. Dr. Kolmer was awarded the 1935 Ward Burdick Medal for his work on poliomyelitis vaccine.

severity and progress of the disease when administered after the onset of paralysis.

MECHANISM OF RESISTANCE AND RECOVERY IN POLIOMYELITIS

While our knowledge of the mechanism of resistance and recovery in poliomyelitis is still incomplete, it would appear that antiviral antibody plays an important rôle. As shown by Schultz, Gebhardt and Bullock[2] this antibody parallels the antitoxins in behavior. Just as soluble exogenous toxins (especially tetanus toxin) are difficult to neutralize with their respective antitoxins once the toxins have attacked cells, so it would appear doubtful if neutralization of endocellular poliomyelitis virus with viricidal antibody can be accomplished, or at least difficult to accomplish. For this reason the treatment of the disease by the administration of antibody contained in convalescent immune serum has yielded but doubtful results when given after the development of paralysis. But is is likely that this antibody may be very helpful in treatment if given sufficiently early in the disease to neutralize virus before the latter has attacked the anterior horn cells of the spinal cord. Since the vaccine appears to produce antibody rather rapidly in some cases at least it would appear that it may possess prophylactic and curative value when administered during the incubation period and early stages of the disease. The results of this study have shown that this is true to some extent in experimental poliomyelitis of monkeys.

TECHNIC AND RESULTS

Adult *Macacus rhesus* monkeys were inoculated intracerebrally under ether anesthesia with 0.5 cc. of a 5 per cent suspension of spinal cord virus which represented from five to ten minimal infective doses producing paralysis of four controls in from 7 to 8 days as shown in the accompanying table.

Twenty-four hours later four of these animals received the first of three daily subcutaneous injections of vaccine in dose of 0.05 cc. per kgm. and equivalent to 3 cc. for a human adult of 60 kgm. Two of these animals remained perfectly well; one developed a slight paralysis of the right arm which did not progress while the fourth monkey developed a severe paralysis on the eighth day which became progressive.

Three monkeys received the first of three daily doses of vaccine three days after intracerebral inoculation. Two remained perfectly well while the third

developed paralysis on the eleventh day after infection which became progressive.

Three monkeys received the first of three daily doses of vaccine 5 days after intracerebral inoculation with virus which was about 2 to 3 days before the onset of paralysis in the controls. One remained perfectly well while the

TABLE 1

RESULTS OF VACCINE TREATMENT

MONKEY	INTERVALS AFTER INFECTION* BEFORE VACCINE WAS GIVEN	VACCINE†	RESULTS
1	24 hours	3 daily doses	Remained well
2	24 hours	3 daily doses	Slight paralysis on 14th day. No progression
3	24 hours	3 daily doses	Severe paralysis on 8th day
4	24 hours	3 daily doses	Remained well
5	3 days	3 daily doses	Remained well
6	3 days	3 daily doses	Remained well
7	3 days	3 daily doses	Severe paralysis on 11th day
8	5 days	3 daily doses	Remained well
9	5 days	3 daily doses	Slight paralysis on 13th day. No progression
10	5 days	3 daily doses	Slight paralysis on 13th day. Progressive
11	8 days; slight paralysis present	1 dose	No progression; survived
12	9 days; slight paralysis present	3 daily doses	Progressive paralysis; killed
13	10 days; moderate paralysis present	3 daily doses	Progressive paralysis; killed
14	12 days; severe paralysis	3 daily doses	No improvement; died
15	Control	None	Paralysis; 7th day
16	Control	None	Paralysis; 8th day
17	Control	None	Paralysis; 8th day
18	Control	None	Paralysis; 7th day

* By intracerebral inoculation with 0.5 cc. of 5 per cent suspension of virus.
† 0.05 cc. per kilogram by subcutaneous injection.

remaining two developed paralysis on the thirteenth day after infection with virus.

In this experiment the incubation period following the intracerebral injection of virus before the onset of paralysis was from 7 to 8 days. Three daily subcutaneous injections of vaccine, however, were effective in preventing the paralysis of the disease in some monkeys when given during the first three days of the incubation period and in the case of one animal were affective when given

as late as on the fifth day. Furthermore, there were no evidences of a "negative phase" or period of increased susceptibility to infection from the vaccine in the dosage administered.

One monkey (No. 11) was given a single dose of vaccine on the eighth day after inoculation or about 24 hours after the onset of slight paralysis of one of the arms. There was no progression of paralysis and the animal lived indefinitely; while it is not possible to state that this result was due to the effects of the vaccine yet the possibility and probability is to be recognized.

In the remaining three monkeys of the series (Nos. 12, 13 and 14) paralysis first appeared in 7 to 8 days after inoculation. Three daily subcutaneous injections of vaccine were started on the ninth, tenth and twelfth days respectively but in all three animals the paralysis was progressive without any demonstrable curative effects on the part of the vaccine.

Under the circumstances it would appear that while the vaccine may have been effective in ameliorating the severity of the disease in one monkey (No. 11) receiving it about 24 hours after the first onset of paralysis, yet it was without demonstrable curative effects when first given 2, 3 and 5 days after the onset of paralysis.

In this connection it is to be remembered that the experimental disease was quite severe and that the results from the vaccine may have been better in less fulminant infections. With this thought in mind we repeated the experiment with a second group of monkeys inoculated with ten daily instillations of virus into the nose but the controls were so irregularly infected that the results cannot be properly evaluated except to state that none of the animals receiving three daily subcutaneous injections of vaccine in the same dose per kilogram of weight during the first 7 days of intranasal inoculation with virus developed the disease.

CONCLUSIONS

1. Further evidence of the rapidity of production of immunity in monkeys by subcutaneous injections of the Kolmer vaccine of 4 per cent remote monkey passage poliomyelitis virus in 1 per cent solutions of sodium ricinoleate has been obtained.

2. Five of a series of ten monkeys given three subcutaneous injections of the vaccine during the incubation period, 1, 3 and 5 days after intracerebral inoculation with five to ten minimal infective doses of virus, escaped paralysis and remained perfectly well.

3. All of four unvaccinated control monkeys developed paralysis in from 7 to 8 days after intracerebral inoculation of virus.

4. One monkey given three doses of the vaccine on the eighth day after intracerebral infection and presenting paralysis of one arm showed no further progression of the disease.

5. All of three monkeys given three doses of the vaccine 2 to 5 days after the onset of paralysis were not improved and developed progressive paralysis.

6. The results have indicated the possibility of the vaccine effectively protecting some monkeys against experimental poliomyelitis when given during the incubation period of the disease.

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QUALITATIVE CHANGES IN NEUTROPHILIC LEUKOCYTES*

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On April 25, 1853, Doctor Hermann Welcker[15] bled Margaret Mueller and found that 12,133 leukocytes per cubic millimeter were present in the blood withdrawn. This first recorded leukocyte count initiated the quantitative study of the white blood cells. Welcker says Margaret appeared to be a "full-blooded" girl who complained of lassitude and headache, and she was bled because her illness "remained puzzling." Her erythrocytes, however, were found to number only 1,909,966 per cm., so Margaret was "full-blooded" by clinical observation only. It seems most probable that her symptoms were due to too little rather than to too much blood. Welcker's experience with this patient emphasized the need for the quantitative study of the blood in the laboratory to supplement clinical observation. In 1879 Ehrlich[3] demonstrated basophilic, acidophilic and neutrophilic granules in the polymorphonuclear leukocytes in stained blood films and he made possible by his method of staining the simple and accurate identification of the different types of leukocytes. Before Ehrlich's observations, different types of leukocytes had been recognized, but little had been learned about them. Even Virchow, the discoverer of leukocytosis, erroneously attributed this condition to an increase in the number of lymphocytes because of his difficulty in differentiating the leukocytes of the blood[4]. Potain[12] invented the blood diluting pipette in 1867 and Gowers[6], the modern counting chamber in 1877. With these improvements and with the advent of Ehrlich's method of staining, the making of differential counts as well as enumerations of the total

* Read before the Fourteenth Annual Convention of the American Society of Clinical Pathologists held at Atlantic City, June 7 to 9, 1935.

number of leukocytes present in the blood were soon utilized with increasing frequency in clinical medicine.

The second advance in the qualitative study of the leukocytes was the attempt of Arneth (1) in 1904 to measure the relative age of the circulating neutrophilic leukocytes from the morphology of the nucleus. Arneth showed that the nucleus of the neutrophil, in its development from the myelocyte in the marrow, becomes gradually more indented, and divides with age into an increasing number of separate lobes or segments. The architecture of the nucleus is thus an index of the maturity of the cell.

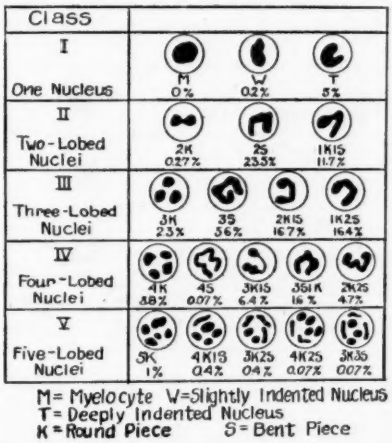

FIG. 1. ARNETH CLASSIFICATION OF THE NEUTROPHILIC LEUKOCYTE

He divided the neutrophils into five major groups and numerous sub-groups on the basis of nuclear configuration (fig. 1). The nucleus in group I has only one lobe and in group V it has five or more lobes. Arneth suggested the terms "shift to the left" to indicate an increase in young cells or in those with fewer lobes, and "shift to the right" to designate an increase in older cells or in those with a larger number of lobes. Arneth's complete classification is unfortunately entirely too complicated for practical laboratory application. His observations on the relation of the nuclear form to the age of cell, however, have been accepted as fundamentally correct, and have stimulated further study and

classification of the maturity of the neutrophils which have proved of great value in clinical hematology.

Schilling[13] in 1911 suggested a classification of the neutrophilic leukocytes (fig. 2) which has been widely employed especially in the study of diseases related to infection. He divides the neutrophils in the order of their age into four groups: (1) myelocytes; (2) juvenile nuclears in which the nucleus has become indented; (3) stab or staff nuclears in which the nucleus is T-V- or U-shaped without division into segments; and (4) segmented nuclears which are fully differentiated neutrophils with distinct segmentation into from two to five lobes. Schilling's classification is much simpler than Arneth's and recognizes two types of "shift to the

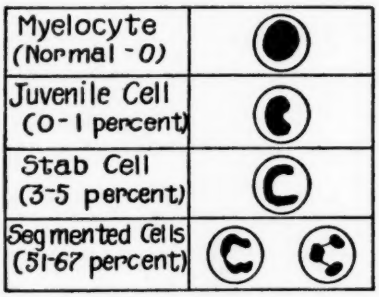

FIG. 2. SCHILLING CLASSIFICATION OF THE NEUTROPHILIC LEUKOCYTE

left." Arneth, in his "shift to the left," considered that the increased number of cells with fewer lobes was caused only by the rapid outpouring of leukocytes from the marrow in response to an acute need before growth and differentiation were complete. Schilling designates this a *regenerative* "shift to the left." In some instances a depression of bone marrow function due to toxins seems to prevent the complete differentiation of the neutrophils, so that they develop only to a certain point and emerge into the circulation at this stage. The differential count in such cases shows an increased number of immature forms due to the depressed marrow function. Schilling designates this type of reaction as a *degenerative* "shift to the left." Other evidences of degeneration of the neutrophils are also seen here, such as loss of structure and narrowing and deep-staining of the nucleus, irregu-

larity in size and staining reaction of the granules in the cytoplasm and the vacuoles.

Schilling in classifying the neutrophils, rightly places the emphasis on the more immature forms. Arneth had emphasized the subdivision of the segmented or more mature types. It is also often difficult to determine when the nucleus of any given cell has become segmented. Cooke and Ponder[2] suggest a simple criterion, which is now known by their names, for determining division of the nucleus. The nucleus never divides completely. The lobes are connected either by a fine filament or by denser bridges of nuclear material. Cooke and Ponder do not classify a nucleus as divided if the segments are

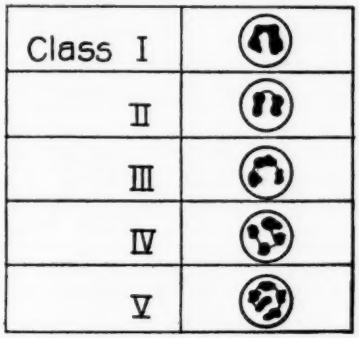

FIG. 3. COOKE AND PONDER CLASSIFICATION OF THE NEUTROPHILIC LEUKOCYTE

connected by other than a fine chromatin thread (fig. 3). By using this criterion of division it is relatively simple to classify all neutrophils in a well-made film. Cooke and Ponder recognize five groups of neutrophils having from one to five distinct lobes in the nucleus. While this is a valuable simplification of Arneth's classification, the emphasis is placed on subdivision of the segmented or more mature cells as in Arneth's method, rather than on the more immature cells. It is desired to know the degree of immaturity of the neutrophils rather than the extent of maturity. Pernicious anemia is one of the few clinical conditions in which the appearance of a larger number of very mature neutrophils or "shift to the right" is of diagnostic importance. Some "shift to the left"

is, however, encountered in the presence of almost every infection, and often in other toxic conditions.

Several observers have simplified still further the grouping of the neutrophils and at the same time they have preserved the essential and valuable features of this procedure. Myelocytes are seen infrequently. Pons and Krumbhaar[11] suggest the division of the neutrophils into three classes: (1) metamyelocytes or very young forms in which there is only a slight indentation of the nucleus (the juvenile form of Schilling); (2) nonsegmented or young

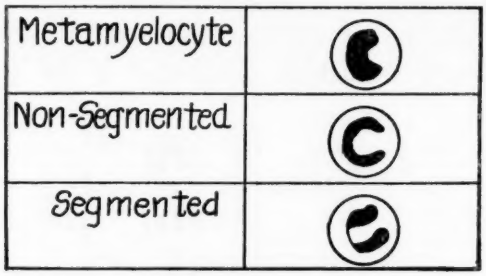

FIG. 4. PONS AND KRUMBHAAR CLASSIFICATION OF THE NEUTROPHILIC LEUKOCYTE

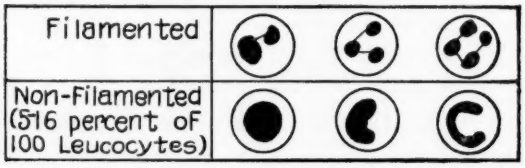

FIG. 5. FARLEY ET AL. CLASSIFICATION OF THE NEUTROPHILIC LEUKOCYTE

neutrophils (stab form of Schilling); and (3) segmented or older cells (fig. 4). These authors do not mention criteria for differentiating segmented and nonsegmented cells although their illustrations show as segmented cells only those in which the lobes are connected by a filamentous thread. Farley, St. Clair and Reisinger[5] suggest the use of Cooke and Ponder's criterion for determining segmentation and the simple division of all neutrophils into filamented and nonfilamented cells (fig. 5). Neutrophils in which two or more lobes are united only by a filament of chromatin material are recorded as filamented cells, all others are classified as nonfilamented.

The classification of neutrophils into filamented and nonfilamented forms is simply, quickly and accurately made and usually supplies most of the valuable information concerning the degree of maturity of the neutrophil. Schilling's classification is valuable when a more detailed study of maturity is required. Arneth's complete classification has been discarded because it is entirely too complicated for practical use. Cooke and Ponder's count usually gives information of little more value than the simple division of all neutrophils into filamented and nonfilamented forms.

In both the Schilling hemogram and the filament-nonfilament count 100 or more leukocytes are counted and the neutrophils are subdivided. When 100 leukocytes of all types are counted, not

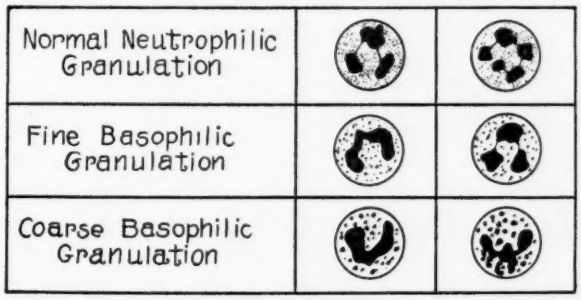

FIG. 6. DIAGRAM TO INDICATE THE APPEARANCE OF BASOPHILIC OR "TOXIC" GRANULATION IN THE NEUTROPHILS

more than 16 per cent of the neutrophils should be nonfilamented. A normal average of neutrophils according to Schilling is found when 100 leukocytes of all types are counted as (1) myelocyte, none; (2) juvenile, 0–1 per cent; (3) stab, 3 to 5 per cent; and (4) segmented, 51 to 67 per cent. In making any of the qualitative counts, a thin, well stained film is necessary. It is far preferable to use only cover glass preparations. The films are stained routinely by the use of Wright's stain and a buffer solution[8].

More recently, qualitative changes in the granules of the cytoplasm of the neutrophilic leukocytes have incited much interest in relation to infection and other toxemias. It has been pointed out by numerous observers[14] that deeply staining, basophilic granules may occur in the neutrophils under abnormal conditions.

The granules in the cytoplasm of normal neutrophils are numerous, small, of uniform size, and pinkish in color. The basophilic granules or "toxic granules," as they are often designated, may be large or small (fig. 6). If the "toxic granules" are small, they are usually distributed among the pinkish neutrophilic granules; if they are large, few if any normal granules are seen in the cytoplasm. The "toxic" granules occur especially in the presence of pneumonia, septicemia, and peritonitis but they may be found in

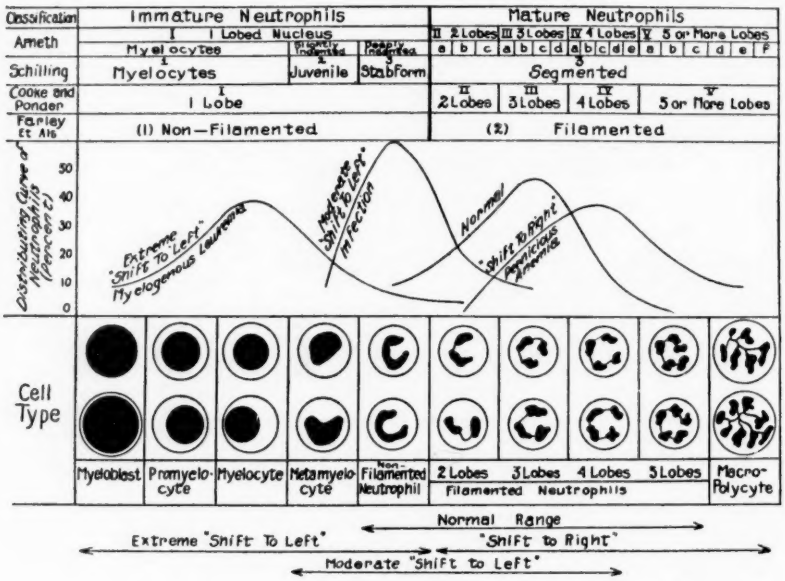

FIG. 7. DIAGRAM TO CORRELATE THE DIFFERENT CLASSIFICATIONS OF THE NEUTROPHIL

any severe infection or toxemia with or without leukocytosis. The granules may be present in either young immature cells or in mature, segmented neutrophils. Large basophilic granules are found only in the acute stage of disease.

In studying cells which show basophilic granulation, one is impressed with the small total number of granules in the cytoplasm. In a normal neutrophilic cell the cytoplasm is packed with small granules. If such a cell is stained by an oxidase

method, the granules are sharply defined and the filling of the cell is evident. Likewise, an oxidase stain on a cell which shows basophilic granulation reveals relatively few oxidase reacting granules (fig. 8). Therefore, the oxidase stain also gives valuable information concerning the number and condition of the granules present in the cytoplasm. Graham[7] pointed out long ago a close relationship between an increase in the younger Arneth forms and a decrease in the number of oxidase reacting granules in the cytoplasm. He likewise showed that conditions such as lobar pneumonia, which is now recognized as a disease in which basophilic

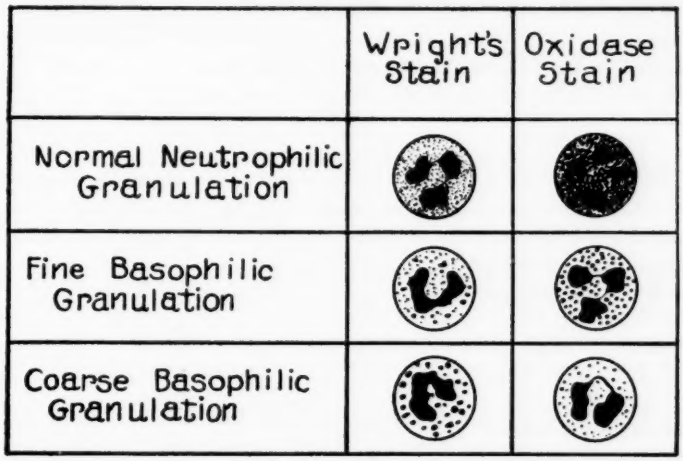

FIG. 8. DIAGRAM TO INDICATE THE RELATION BETWEEN BASOPHILIC GRANULATION AND THE OXIDASE REACTION

granulation is almost uniformly present, exhibit also a great decrease in oxidase granules, although he did not mention basophiiic granulation in this regard. He considered a decrease in the granules as evidence of the presence of a toxic condition. Basophilic granulation, then, really represents granule failure in the same manner as does a decrease in oxidase staining granules.

Basophilic or "toxic" granulation in the neutrophils is looked upon as a phenomenon of degeneration. It seems most probable that it represents the effect of the toxemia on the cells in the marrow at the stage of granule formation. The percentage of

cells which show basophilic granulation may be recorded, or the degenerative index suggested by Kugel and Rosenthal[9] and based on the percentage of neutrophils showing basophilic or "toxic" granules may be used. The index is calculated by dividing the number of neutrophils showing basophilic granulation by the total number of neutrophils counted. A differential leukocyte count which measures satisfactorily the extent of immaturity of the neutrophils as well as the frequency of basophilic or "toxic" granulation is shown in table 1.

In addition to changes in the granules, the staining reaction of the cytoplasm also often differs from that of normal. The

TABLE 1

	TOTAL	NORMAL	BASOPHILIC GRANULATION
	per cent	*per cent*	*per cent*
Polymorphonuclear neutrophils, filamented	60	12	48
Polymorphonuclear neutrophils, nonfilamented	25	5	20
Polymorphonuclear eosinophils	0		
Polymorphonuclear basophils	0		
Lymphocytes	10		
Monocytes	5		

$$\text{Degenerative index} = \frac{\text{Number of neutrophils showing basophilic granulation}}{\text{Total number of neutrophils}} = \frac{68}{85} = 80$$

Percentage of neutrophils showing basophilic granulation.... = 80

cytoplasm may take on a bluish hue of varying intensity which is due to increased basophilia. This diffuse basophilic reaction together with "toxic" granulation occurs frequently. Likewise the pattern of the nucleus may vary from normal with irregularity in staining due to abnormal variations in density and staining reaction.

Basophilic granules and variations in the staining reaction of the cytoplasm and the nucleus may be seen satisfactorily only on thin films which are properly stained. If cover glass preparations are used, control preparations of normal blood should be stained and examined at the same time. If the blood film is made on a

slide, a film of normal blood may be spread on one end of the slide and the two films stained at the same time. Variations in the unknown blood are significant only if they are absent from the normal film. It is important in studying all qualitative changes in the neutrophils to examine serial preparations because much more is learned from a comparison of counts made from day to day or time to time than is learned from single counts.

SUMMARY AND CONCLUSIONS

The qualitative changes in the cytoplasm and nuclear structure of the neutrophils which occur in the presence of disease have been reviewed.

The differential count which measures the extent of immaturity rather than the degree of maturity is the one most valuable.

The filament-nonfilament count of the neutrophils is very simple and usually gives adequate information concerning the extent of immaturity of the neutrophils.

The hemogram of Schilling is practical and also more detailed, but it gives little more information than the simpler counts.

Basophilic or "toxic" granulation indicates the presence and severity of a toxemia; when recorded as the degenerative index valuable data are added to the blood count.

When basophilic granulation is present in neutrophils, there is a decrease in oxidase staining as well as a decrease in the size and staining reaction.

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DIFFUSE DECIDUAL HYPERPLASIA OF THE ENDOMETRIUM IN THE ABSENCE OF PREGNANCY*

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Decidual hyperplasia under normal conditions is fundamentally associated with pregnancy. This hyperplasia may vary considerably in amount within normal limits, but usually reaches its maximum development in the fourth month, when it is approximately 1 cm. in thickness.

Although usually associated with pregnancy, such is not necessarily the case; a demonstration of decidual hyperplasia being insufficient for a diagnosis of pregnancy. Slight transformations of "the cytogenic stroma" of decidual types are occasionally demonstrable in the premenstrual phase of the endometrium. They are not believed to be an integral part of the premenstrual phase of the endometrium as revealed by study of material obtained by the "punch biopsy" method as well as by study of uteri removed from one to three days before menstruation.

Occasionally, either with or without demonstrable pregnancy, a more marked degree of decidual hyperplasia than that which occurs normally during pregnancy is encountered. This has been called "diffuse hyperplastic, polypoid or tuberous decidual endometritis." The cause of this hyperplasia has been ascribed to a "chronic hyperplastic or gonorrheal, or as is sometimes assumed, a syphilitic endometritis before the occurrence of pregnancy, or if it developes during its course[4]." Another type of decidual hyperphasia in the absence of demonstrable pregnancy has been described as the "decidua of menstruation[1]." Diffuse polypoid decidual endometritis is manifestly a composite anatomical de-

* Read before the Fourteenth Annual Convention of the American Society of Clinical Pathologists held at Atlantic City, June 7 to 9, 1935.

scription, while "decidua of menstruation" reflects the profuse some-what periodic discharge seen clinically. It seems probable that these are different pathological manifestations with the same fundamental etiology.

Still another type of uterine decidual hyperplasia in the absence of uterine pregnancy is that associated with ectopic pregnancy. Under these conditions (i.e. ectopic pregnancy), uterine decidual hyperplasia is not always demonstrable[6]. Finally decidual hyperplasia is encountered following "very early abortions (four to five weeks) where the ovum may be expelled and the decidua return again to the uterine mucosa" under which condition it may be associated with a persistent corpus luteum[3].

The production of decidual hyperplasia in experimental animals demands traumatic stimulation of an estrin sensitized endometrium, followed by prolonged progestin stimulation. First produced in pregnant guinea pigs by Loeb[5], later in pseudo-pregnant rats by Allen[2], and finally with extracts of estrin and progestin[7], the necessity of pregnancy as an etiological factor in the production of decidual hyperplasia in experimental animals has been eliminated. The crucial test in man will come with the preparation of an extract of sufficient potency to produce prolonged progestin stimulation. Clinical, histological and experimental evidence seem to indicate a definite relationship between prolonged progestin stimulation and decidual hyperplasia. Certainly such a relationship occurs normally during pregnancy. The question is whether it ever occurs pathologically without a preceding pregnancy. The relation of a persistent corpus luteum and endometrial hypertrophy has been discussed by Pratt[8]. The following case is believed to illustrate diffuse decidual hyperplasia without pregnancy.

CASE HISTORY

A white, married woman, 25 years of age was admitted to the Hospital on the service of Dr. P. J. Reel, with a complaint of a prolonged, profuse (menstrual?) discharge of approximately 10 days duration at each period. This condition had existed for three years and followed the birth of her only child. Following the delivery of this child, a retained placenta was encountered, which required manual extraction. During the past three months the discharge

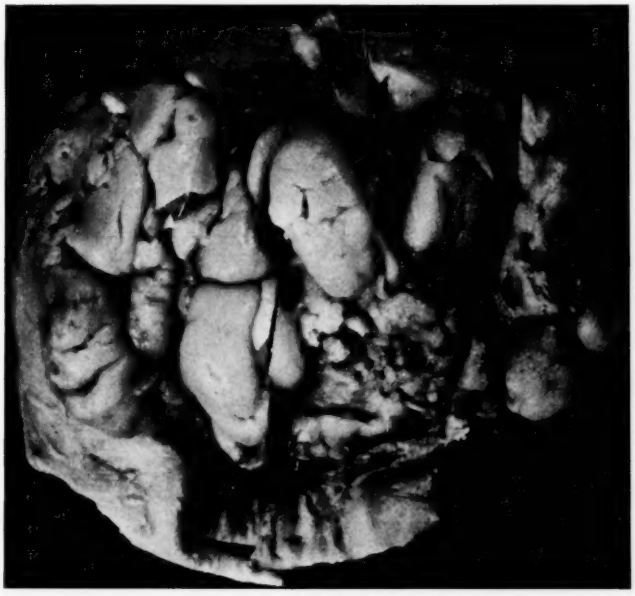

FIG. 1. DIFFUSE POLYPOID DECIDUAL HYPERPLASIA

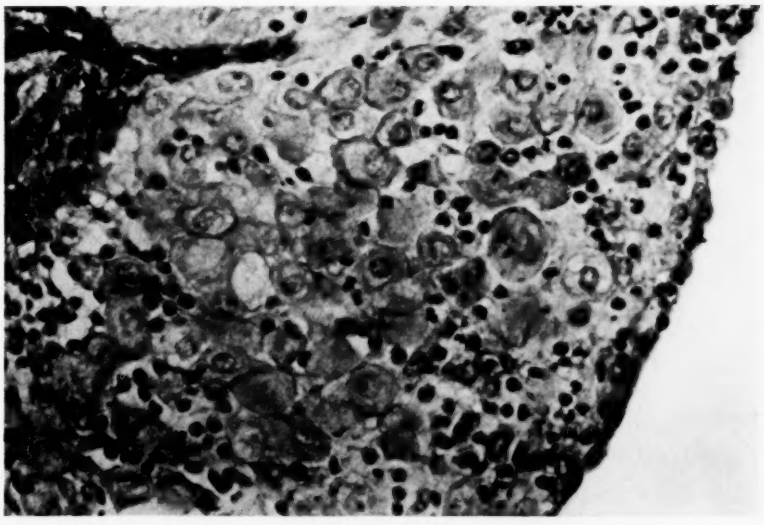

FIG. 2. MARKED HYPERPLASIA OF DECIDUAL CELLS

became more profuse and prolonged and was attended by cramps. The discharge contained less blood than normal, and most of the time was definitely white. There had been no period of flooding. Her last menstrual period of 12 days duration, ended 14 days before her operation.

At operation a "chronic cervicitis with erosion, relaxed anterior vaginal wall, small cystocele and acute retroflexion of the fundus was noted. The fundus was retroverted to the base of the cul-de-sac, of a reddish-purple color, soft, boggy and when returned to its normal position immediately began to blanche. The ovaries were edematous, and there was a large corpus luteum in the right ovary. The tubes were not remarkable. A supravaginal hysterectomy was performed with excision of the upper cervical canal."

The specimen

The body of the uterus slightly enlarged, measured 7 cm. x 5 cm. x 5 cm., and weighed 100 grams. The cervix was not present. The hypertrophy of the body of the uterus was smooth, without nodularity and practically spherical. The peritoneal coat was not remarkable. Upon opening the uterus, thick velvety folds presenting varying shades of red, welled out through the incised muscularis (fig. 1). These folds of endometrial hypertrophy were soft in character, without evidence of hemorrhage or ulceration. There was no gross evidence of necrosis or placental formation.

Microscopic examination revealed a marked hyperplasia of the endometrium, this hyperplasia being almost exclusively the result of a diffuse transformation of the "cytogenic stroma" into decidual cells (fig. 2). The decidual hyperplasia composed the outer half of the endometrium, while the inner half was composed of glandular tissue of premenstrual type, somewhat simulating the so-called "Opitz pregnancy glands."[4] There was a slight infiltration of polymorphonuclear leucocytes and round cells, moderate congestion, very little hemorrhage or necrosis. The absence of necrosis, hyalinized arteries, dilated vessels and hemorrhage stood out in marked contrast to usual cases in which chorionic villi are found. There was no evidence of chorionic villi. However, on the basis of the decidual hyperplasia a blood estrin assay was made by the Frank-Goldberger method, which was negative.

A tentative diagnosis of diffuse decidual hyperplasia without evidence of pregnancy was made.

DISCUSSION

Weller[9], citing the reports of Shiller and Schereshewsky concerning the incidence of decidual formations in the apparent absence of pregnancy raised the question of whether such cases are another manifestation of "decidua menstrualis" and comparable to the premenstrual changes in the endometrium or that the fact of pregnancy has escaped detection.

The histological pattern and clinical history, of this case suggest the diagnosis of "decidua menstrualis." Comparison of this case with three others in which diffuse decidual hyperplasia was present, one with chorionic villi, and two without demonstrable villi, but with clinical histories suggestive of early abortion, indicate a common etiology. The recognition of this type of pathology as one which is dependant upon prolonged progestin stimulation, usually following an early abortion, with persistently active corpus luteum, but possible with any hormonal imbalance which maintains an active corpus luteum, would seem to be justified.

Clinically the outstanding symptom of this case, was the periodically recurring, profuse, prolonged, white discharge with very little blood. The prolonged profuse white discharge which occurs following early abortions is usually constant in character. Histological examination of this case presents little evidence of the mechanism by which the discharge was produced. However, the uterus was removed at a time during which there was clinically little discharge present. Whether this discharge was the result of the involution of the corpus luteum, or a local nutritional inadequacy associated with the rapid tissue growth has not been determined. If the involution of the corpus luteum is the mechanism by which this discharge is brought about, the diagnosis of "decidua menstrualis" might be competent. Histological examination of hyperplastic decidual tissue removed in the cases cited above, during the discharge presents a similarity to the early phase of menstruation.

SUMMARY

(1) A case of diffuse, polypoid decidual hyperplasia in the absence of demonstrable pregnancy is presented.

(2) From the evidence available, the diagnosis of "decidua menstrualis" seems justified.

(3) A condition of prolonged progestin stimulation is suggested as the fundamental etiology.

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THE INTERPRETATION OF THE HISTOLOGIC FINDINGS IN ENCEPHALITIS CONGENITA*

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Fetal tissues in a state of incomplete embryologic development and adult organs which are in a state of functional hyperplasia frequently offer difficulties in the correct interpretation of the histologic alterations, particularly when associated with true pathologic processes.

This is the case when the cerebrum of the premature or newborn term infant is subjected to gross and microscopical examination. Most are prone to examine such a subject for evidence of gross maldevelopment or gross intracranial hemorrhage, and specimens taken for microscopic study are apt to be selected in a haphazard manner. Frequently, therefore, when sections which include the lateral ventricles are studied, one becomes interested in round cell infiltrations, often in perivascular position, beneath the ependyma. When this is associated with perivascular hemorrhages and degenerative changes of variable degree, many pathologists are convinced that the criteria of inflammation have been established, and a diagnosis of encephalitis is made. Such a conclusion seems to be confirmed when the clinical or necropsy data indicates congenital syphilis or postnatal infection. It seems entirely logical to correlate the subependymal cellular features with the established infection. However, such a conclusion has a major factor of error if one does not appreciate the extent, the location and degree of developmental activity in the case under consideration.

The American medical literature is remarkably lacking in references to the subject of encephalitis congenita. Most of the

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contributions to the subject have come from Europe. In 1866 Rudolph Virchow[8] described the condition which he termed "encephalitis congenita" as a combined inflammatory and degenerative process in the white substance of the cerebrum. He placed particular stress on the importance of the fatty degenerative changes which were associated, and explained the pathogenesis by an attraction theory wherein the medullary substance of the brain, under stimulation of the inflammatory process, becomes more active, absorbs more nutriment, and the cells increase in size and number and ultimately progress into a regressive stadium which results in fatty metamorphosis. Virchow believed that the acute exanthemata, particularly syphilis, were etiologic factors. The emphasis placed upon the importance of the fatty changes created a sharp controversy regarding the significance of such changes. Von Graefe[10] substantiated the findings of Virchow. Jastrowitz[3] did not entirely disagree with the teaching of Virchow, but he regarded the granular fatty cells in the brain of the new-born infant as having no pathologic significance, stating that the presence of these cells is a physiologic manifestation from the fifth intrauterine to the seventh to eighth extrauterine months, since he found these fatty cells in the brains of all infants examined. He held the view that the fatty glial cells furnished the material for the development of the medullary sheaths. Schroeder[7] stated that the glial cells produced a large number of granular fatty cells during developmental and degenerative changes in the central nervous system.

Lubarsch[5] questioned the inflammatory character of such fatty changes, insisting that an inflammation should be manifested by alterative, exudative and productive changes in variable degree.

Ceelin[1] stated that from the present day knowledge of the nature of inflammation, the criteria submitted by the supporters of Virchow's idea are not valid since in the main the process is concerned with alterative changes in the glial cells and axis cylinders. It is noteworthy that Virchow[9] in a later publication questioned the complete accuracy of his opinion, but stated that if the process was not an inflammation in the strict sense of the word, it was nevertheless an irritative process which lead to fatty metamorphosis.

Throughout this controversy it is well to remember that most of the investigators described perivascular inflammatory infiltration in the white substance of the brain. Ceelin, who has made the latest complete report on encephalitis congenita, stresses the exudative features in his series of twenty cases, and attempts to associate the etiology with purulent omphalitis which was present in several of his cases. In order to support such an etiology in the remainder of his group, he offered the explanation that tetanus in the new-born does not necessarily show inflammation of the umbilicus, but that organic changes in the central nervous system are the basis of the disease.

P. Manunza[6] suggested malaria as a possible etiology in two cases which he reported, but enumerated the entire gamut of infectious diseases as causes to be considered. It is noteworthy that both of his cases were prematurely born infants.

When considering an interpretation based on inflammation as a cause of the microscopic picture of a case which Ceelin considered to be caused by a pneumococcus infection of the umbilicus, it is well to carefully analyze his description.

> In the white substance about the lateral ventricle one sees a marked congestion. Blood vessels contain numerous polymorphonuclear cells. In the perivascular lymph spaces there is a prominent accumulation of deeply stained round cells mainly of the character of lymphocytes. This perivascular infiltration does not accompany the vessel throughout its course, but is largely local and to one side. Occasionally it surrounds the vessel. The intervening brain substance is extraordinarily cellular. Elongated leucocytes and small, intensely stained lymphocyte-like cells are found with larger palely stained cells, which doubtless are proliferating glia cells. Some of these contain fat droplets and some show nuclear division forms. The cerebral cortex and central radiations, as well as the cerebellum, pons and medulla, are free from these changes. In this case we have the typical picture of encephalitis manifested by alterative, exudative and productive changes. The site of predilection for such alterations is the subependymal medullary layer of the posterior and inferior horns of the lateral ventricle.

This description may be taken as a typical example of the histologic features which are utilized to substantiate the diagnosis of encephalitis congenita. Examination of the brains of new-born infants indicates that such features are largely, if not entirely,

evidence of incomplete cerebral development The cytologic appearances in such a brain are deceptive because of the suggestive infiltrative and exudative character of the changes. The invasive and migratory phases of cerebral cellular development has been described by Kappers[4] who used the term "neurobiotaxis" in his explanation of this process.

I have encountered several instances of outstanding cellular infiltration with suggestive perivascular inflammatory cell mantles forming a granulomatous appearing mass beneath the ependyma of the lateral ventricles in new-born infants. One such case was associated with an acute appendicitis in the mother and periappendiceal abscess which developed just before labor. It seemed logical at the moment to assume that the fetal cerebral change was an encephalitis due to maternal pyogenic infection.

Four of my cases were discovered when ventricular sections were taken from the brains of new-born infant which exhibited gross ventricular hemorrhage, and at first, it appeared that fetal encephalitis was a factor in the causation of such hemorrhages.

In an effort to investigate the etiologic and pathologic features of such cases I studied the material in the files of the University of Michigan Hospital with the kind coöperation of Dr. Carl Weller. I found thirty-two cases which exhibited in some degree the cellular features of encephalitis. Taking into consideration the fact that such changes were found in routine sections cut without regard to establishing localization of the lesions, it is significant that the microscopic changes were always situated in the subependymal medullary tissue of the lateral ventricle, while the remainder of the brain, the adjacent choroid plexus and the meninges showed no inflammatory reaction. Moreover, the other fetal or infant organs showed no inflammatory changes, except in a few cases which presented evidence of congenital syphilis or where the infant lived a short time and developed pneumonia. The maternal histories were then studied, and in only eight of the thirty-two cases was there any direct or indirect evidence of infection during the pregnancy in question.

A summary of the data on these eight cases is contained in table 1.

This tabulation indicates that the subependymal cellular reaction is most prominent in the more premature infants. The apparent exception to this statement is case 3. This is explained

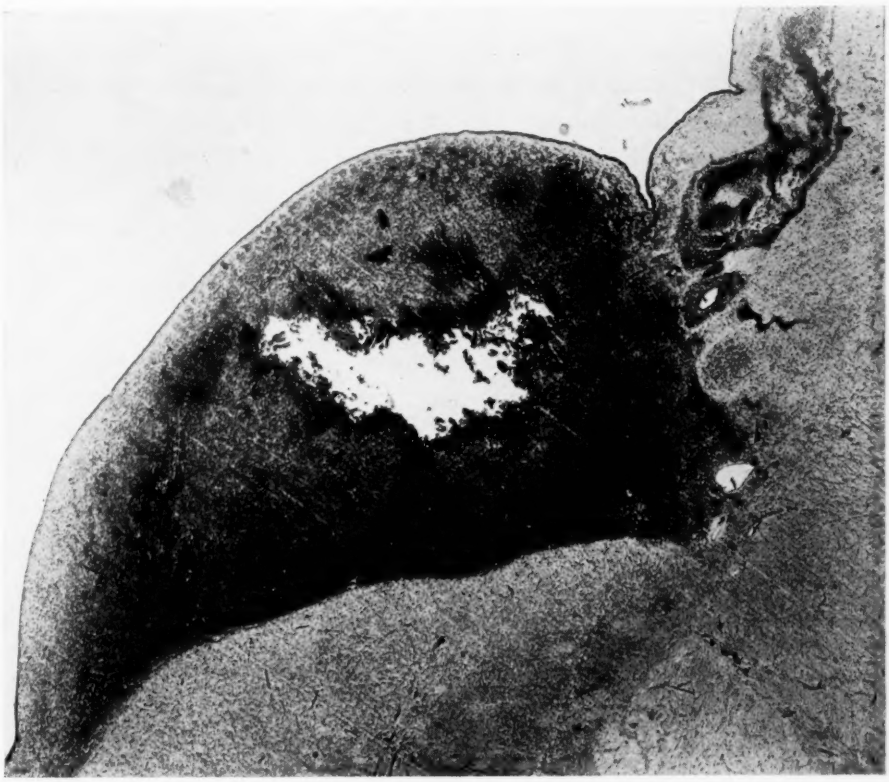

FIG. 1. THE LARGE SHARPLY DEFINED GRANULOMATOUS-APPEARING MASS OF NEUROGENIC CELLS IN THE ANGLE BETWEEN THE CAUDATE NUCLEUS AND THALAMUS OF A 32-WEEK FETUS

The perivascular mantles are seen in the upper right quadrant. Central hemorrhage and tissue destruction. (Magnification: 32 mm. microtessar; 50 cm. bellows length.)

by the fact that a single section was taken to include a very small area of the ventricular surface, and this section did not give a true picture of the subependymal conditions.

Case 4, a term infant, showed a slight subependymal reaction, and case 1, a slightly premature infant, which lived one month, showed a few scattered round cells.

In the twenty-four infants, with birth development ranging from 18 weeks to full term, born of mothers who gave no evidence of infection during pregnancy, it was noted that the most prominent subependymal cellular reaction was seen in the premature

TABLE 1

SUMMARY OF EIGHT CASES

CASE NUMBER	TYPE OF CASE	FETAL DEVELOPMENT	DEGREE OF NEUROGENIC ACTIVITY
1	Serologic evidence of syphilis	Slightly premature, 8½ months. Lived 1 month	Occasional scattered cells
2	Serologic evidence of syphilis	Premature, 35 weeks	Moderate, over basal ganglia
3	Serologic evidence of syphilis	Premature, 28 weeks	Slight, only one ventricular section
4	Infected teeth	Term	Slight, local, perivascular
5	Infected teeth	Development not noted; lived 3 days. Died of pneumonia	Prominent local subependymal aggregates and perivascular mantles
6	Infected teeth. Nasal sinusitis	Premature, 28 weeks	Prominent granulomatous and diffuse reaction. Slight perivascular mantles
7	Gonorrhea. Organisms demonstrated	Premature, 33 weeks	Prominent cellular reaction
8	Chronic pelvic inflammatory disease	Premature, 18 weeks	Prominent reaction, over basal ganglia

group. The location and character of the reaction in this larger group was no different than that seen in the group born of mothers who had infection.

In order to remove the factor of chance sectioning, and to determine the extent and degree of this cellular reaction in relation to estimated fetal development, a series of brains of new-born infant was subjected to uniform cross-sectioning throughout the entire

ventricular system. At present this series consists of forty-six brains, ranging in fetal developmental age from 12 weeks to term.

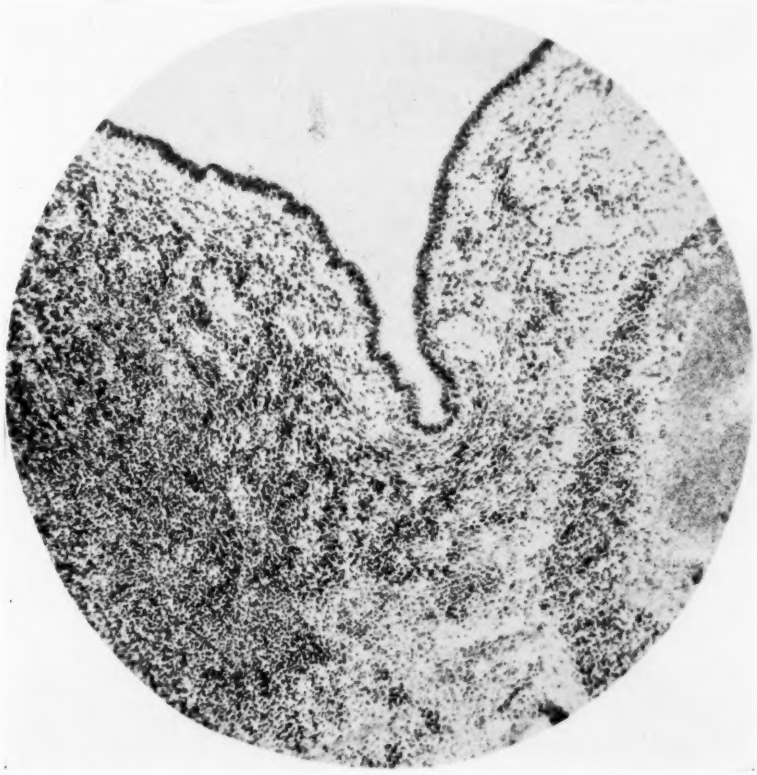

FIG. 2. AREA NEAR THE SLIGHT VENTRICULAR RECESS SHOWN IN FIG. 1

Perivascular mantle at the right. Hemorrhage and tissue destruction at the left. Small aggregates of intensely stained small round and slightly elongated cells in the center. (Magnification: 16 mm. obj.; 7.5 occ., 55 cm. bellows length.)

None of these infants survived longer than 48 hours after birth. The following conclusions were drawn from this study:

(1) Every brain in the series exhibited some degree of subependymal cellular activity. The degree of activity was proportional to the degree of hypodevelopment. The nature of the

reaction was in no manner different in location, extent, or cellular character from that seen in cases interpreted as encephalitis congenita.

(2) At 12 weeks the neurogenic activity was present in the wall of the entire lateral ventricle system with a prominent mass of undifferentiated cells overlying the developing basal ganglia. The cells had small, round, intensely stained nuclei with little cytoplasm. They were closely assembled in a finely vascularized stroma with a slight tendency to group into aggregates, but no perivascular mantles were formed.

(3) At 16 weeks the neurogenic activity was still evident about the entire lateral ventricular system, the mass of cells over the developing basal ganglia was large, and it formed a distinct projection into the lateral ventricle. Most of the cells were small, round and intensely stained, but some were distinctly elongated and showed short protoplasmic processes. There was a slightly greater tendency to form cell groupings. The stroma was rich in capillaries, but no perivascular mantles were seen. At the lateral and inferior margin of this mass strands of cells extended outward into the medullary tissue of the internal capsule. The cell mass attained its greatest thickness back of the foramen of Monroe, and tapered off until it merged with the diffuse narrow zone of cells beneath the ependyma of the posterior part of the lateral ventricle. The cells unquestionably were concerned in the process of producing undifferentiated elements for the development of the caudate nucleus, the thalamus and the interganglionic communication tracts.

(4) At 21 weeks the cell mass still formed a thick, cellular layer over the basal ganglia and had extended more posteriorly. There was a greater tendency to the formation of somewhat larger round cells and areas where cells with small round nuclei were less closely assembled in a delicate fibrillary reticulum. There was still no formation of perivascular mantles, but the more deeply situated blood vessels were larger and more distinctly formed.

(5) At 26 weeks the cell mass was still prominent over the basal ganglia, and it formed a distinctly outlined area projecting into the lumen of the lateral ventricle. Many cells were larger

and many were slightly elongated. Some distinct foci in the outer zone consisted of a fibrillary stroma with very small round cells. There unquestionably were fiber tracts. No perivascular mantles were seen. The reaction on the median wall of the lateral ventricle was greatly lessened, while a thin zone of activity existed on the lateral wall of the posterior and inferior cornua.

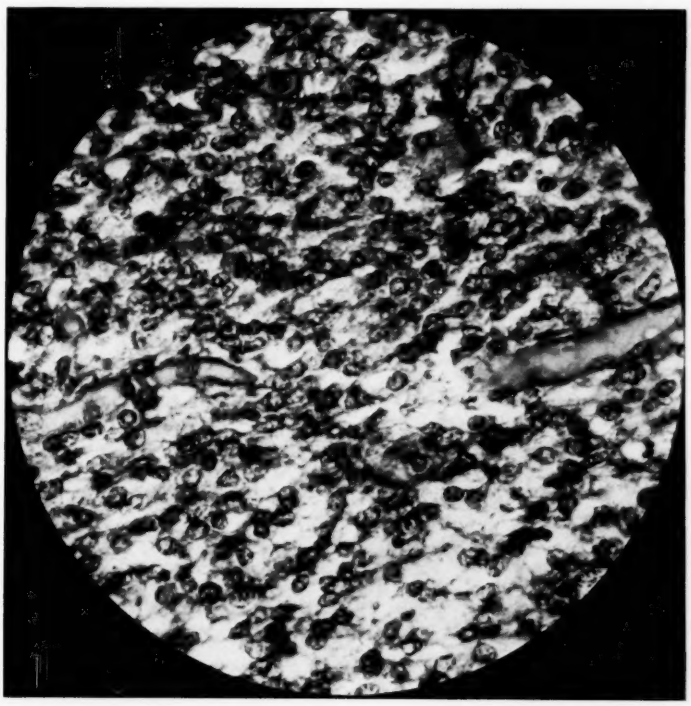

FIG. 3. GLIOBLASTOMA IN THE NEW-BORN

(Magnification: 16 mm. obj.; 7.5 occ.; 55 cm. bellows length)

(6) Throughout these stages of development no neurogenic activity was noted about the third ventricle, and the aqueduct. Only slight activity was noted in the fourth ventricle of the 12 week embryo.

(7) At 29 weeks the zone of neurogenic activity was reduced to a layer of cells about 1 mm. in thickness overlying the basal ganglia and in cross section of the brain extended from the inferior

angle of the lateral ventricle half way up the lateral wall and tapered off into a narrow zone to again become somewhat more prominent at the upper angle of the ventricle. A section, through the frontal lobe including the olfactory lobe, showed a zone of similar activity about the olfactory ventricular process, and in the center of the posterior part of the olfactory nerve. Sections taken more posteriorly in the lateral ventricle, but anterior to the curvature into the descending horn, showed a concentration of primative cells in the angle between the thalamus and caudate nucleus. At this stage of development many distinct perivascular mantles were seen, particularly at the superior and inferior angles of the lateral ventricle and at the angle between the thalamus and caudate nucleus. These mantles occasionally completely encircled the blood vessel, but more frequently were in a sector position. The cells were usually of round type, larger than the more primitive cell seen in the earlier stages of development, but many of them were short spindles and had unipolar protoplasmic processes. At times these aggregates of deeply stained cells were found a short distance from a blood vessel, and at the angles of the ventricle extended out into the medullary tissue. The reaction on the median wall of the lateral ventricle was very slight and some perivascular mantles were seen.

(8) At successive stages of development the reaction progressively receded until at term the neurogenic activity consisted of a few small round cells beneath the ependyma of the lateral ventricle, its anterior horn and, to a very slight degree, in the lateral wall of the posterior horn. Sections which included the angle between the caudate nucleus and thalamus showed a triangular area of undifferentiated neurogenic cells and often some round cells about the blood vessels.

It is this cellular reaction which frequently is interpreted as being inflammatory, particularly in cases of prematurity or when other evidence of fetal or infant infection exists. It is my opinion that most of the cases diagnosed as encephalitis congenita are not examples of cerebral inflammation, or, if inflammation does exist, the accompanying description of tissue alterations is largely concerned with features of late fetal cerebral development. This

applies both to the lipoid and cellular proliferative features of the microscopic picture.

That such developmental features may in instances have an association with cerebral pathology found at or shortly after birth is suggested in those cases which exhibit intraventricular hemorrhage. My attention was directed to this possible factor in my four original cases where prominent neurogenic activity was found in prematurely born infants which exhibited massive intraventricular hemorrhage due to rupture of the blood vessels in the cap of neurogenic tissue overlying the basal ganglia. Gross ventricular hemorrhage was found in eight of the subsequent seventy-eight brains examined, and seven of these occurred in premature infants. Gross focal hemorrhage was found in the neurogenic cap in the 12 week fetus. Microscopically, punctate hemorrhage and perivascular hemorrhagic extravasation in the zone of neurogenic tissue was found in approximately 25 per cent of the entire series; with but a few exceptions this occurred in premature infants. It is evident that the very soft proliferating tissue with its delicate vascular bed cannot easily withstand the vascular changes incident to labor. Prematurity is associated with distinct hemorrhagic liabilities. The attendant destruction of neurogenic tissue and interference in further development of ganglionic tissue and association tracts probably plays an important part in subsequent structural and functional cerebral pathology when the individual survives the birth trauma.

The relative frequency with which isolated ependymal epithelial inclusions were found near the lateral and fourth ventricles and the possibility that some of the undifferentiated neurogenic cells may persist as such, suggests that these elements may give rise to gliomatous tumors in early childhood. That the regulation of development control may go awry during fetal development and result in exuberant proliferation of certain cellular elements is suggested in one of the cases where massive hemorrhage involved the region of the thalamus at birth and sections from this area reveal a tumorous growth of large cells with vesiculated nuclei in an excessive rich vascular bed, the entire picture being that of a glioblastoma. This is a very unusual condition, there

being but one other report of a glioma at birth in the literature, that by Hemsath and Canavan[2].

CONCLUSIONS

(1) Subependymal infiltration of small round cells and perivascular mantles of such cells, found in the brain of the newborn, are not to be interpreted as an expression of inflammation. It is definitely developmental in nature.

(2) This reaction is most pronounced in the prematurely born infants. Between the 12th and 28th week of fetal development this reaction forms a prominent mass over the basal ganglion beneath the lateral ventricle.

(3) The most prominent exhibit of the incomplete state of cerebral development in the term or near term infant is observed in the angle between the caudate nucleus and the thalamus and at the upper lateral angle of the lateral ventricle in sections through the anterior half of the brain.

(4) Failure to properly interpret such manifestations rests upon an incomplete understanding of cerebral development, especially in relation to the formation of the neo-striatum and internuclear pathways.

(5) Incomplete cerebral development at birth offers a distinct liability to cerebral and ventricular hemorrhage during birth, and to late hypodevelopment, gliosis and possible gliomatous tumor formation in later life.

(6) It is admitted that encephalitis congenita per se can exist, but such a diagnosis must depend upon the presence of a true inflammatory reaction; cellular changes which are associated with development must be excluded from the criteria supporting such a diagnosis.

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STUDIES OF PATHOLOGICAL BODY FLUIDS: THE CHOLESTEROL PARTITION AND THE TOTAL PROTEIN CONTENT*

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The cholesterol and protein concentrations in pathological body fluids have been investigated by a number of workers; Gorecki,[6] Bezançon, Weil and Guillaumin,[1] Foord, Youngberg and Wetmore,[5] and more recently, Man and Peters[7] have shown that in such fluids the total cholesterol and total protein contents parallel one another. In the present study, the free and ester cholesterol in relation to the protein content of such fluids and to the cholesterol partition in the plasma have been investigated; in addition, the observation of the writers cited above in regards to the relation between the cholesterol and protein contents of pathological body fluids has been verified. A serial study of a number of pleural exudates in a patient with adenocarcinoma of the lung indicated to what extent the human organism can be deprived of cholesterol through abnormal secretions.

MATERIAL AND METHODS

Thirty-one pathological body fluids (pleural and ascitic fluids of fifteen patients) were analyzed for free and ester cholesterol and for total protein. Prior to chemical determinations the fluids were filtered through fat-free filter paper in order to remove fibrin clots and suspended cells. In thirteen instances, simultaneous studies of the cholesterol partition in the plasma were made. The type of fluid, the clinical diagnosis and the number of fluids investigated are summarized in table 1.

The cholesterol partition in body fluid and in plasma was determined by the Bloor-Knudson[4] method. Colorimetric estimations for cholesterol were carried out according to the modified procedure used in this laboratory.[8] The total protein in body fluids was determined according to the method of Wu and Ling[14] as described by Peters and Van Slyke.[9]

* Aided by a grant from the Josiah Macy, Jr. Foundation.

RESULTS

Table 2 shows the total cholesterol and total protein contents of nineteen pathological body fluids and the simultaneous determination of the plasma cholesterol in thirteen instances; the cases are arranged according to increasing concentration of cholesterol in the effusates. Although no absolute relationship exists between the cholesterol and protein contents of such fluids, it is apparent that the cholesterol more or less parallels the protein concentration. The transudates in the first eight cases show low

TABLE 1

TYPE OF FLUID	DIAGNOSIS	NUMBER OF FLUIDS STUDIED
Pleural	Adenocarcinoma of lung	11
Pleural	Cardiac decompensation	5
Pleural	Pulmonary tuberculosis	3
Pleural	Mediastinal lymphosarcoma	1
Pleural	Lobar pneumonia	1
Ascitic	Toxic cirrhosis of liver (carbon tetrachloride poisoning)*	3
Ascitic	Adenocarcinoma of the ovary	2
Ascitic	Syphilitic cirrhosis of liver	2
Ascitic	Lipoid nephrosis	2
Ascitic	Primary carcinoma of the gall bladder with omental metastases	1

* The clinical aspects and post mortem findings in this case were recently reported by POINDEXTER, C. A., AND GREENE, C. H.: Toxic cirrhosis of the liver. Jour. Am. Med. Assn., **102**: 2015, 1934.

cholesterol and low protein contents; the exudates in the latter seventeen, fairly high cholesterol and high protein concentrations. The observation of Man and Peters that no uniform relationship exists between the concentrations of cholesterol in body fluid and in plasma is confirmed. In none of the cases did the cholesterol content of the effusate exceed that of the plasma, although in some instances, the fluid cholesterol approached rather closely that of the blood. Exceptions to this observation have been recorded, as will be discussed later.

Table 3 indicates the total and ester cholesterol in effusate and

in plasma carried out simultaneously in seven instances. This protocol demonstrates that although at times the cholesterol

TABLE 2

SIMULTANEOUS DETERMINATIONS OF THE CHOLESTEROL AND PROTEIN CONTENTS OF PATHOLOGICAL BODY FLUIDS AND THE CHOLESTEROL CONTENT OF THE PLASMA

CASE	DIAGNOSIS	TYPE OF FLUID	FLUID		PLASMA
			Total cholesterol	Total protein	Total cholesterol
			milligrams per cent	*grams per cent*	*milligrams per cent*
1	Nephrosis	Ascitic	Trace	0.02	422
2	Nephrosis	Ascitic	4.5	0.29	605
3	Toxic cirrhosis of liver. CCl_4 poisoning	Ascitic	13.3	0.90	
3	Toxic cirrhosis of liver. CCl_4 poisoning	Ascitic	14.6	1.01	
3	Toxic cirrhosis of liver. CCl_4 poisoning	Ascitic	22.3	0.96	195
4	Cardiac decompensation	Pleural	29.4		110
5	Syphilitic cirrhosis of liver	Ascitic	31.3	1.57	
6	Cardiac decompensation	Pleural	35.8		211
7	Lobar pneumonia	Pleural	49.0	4.81	
8	Pulmonary tuberculosis	Pleural	53.2		186
9	Primary carcinoma of gall bladder with omental metastases	Ascitic	56.8	3.33	141
10	Adenocarcinoma of ovary	Ascitic	80.8		215
5	Syphilitic cirrhosis of liver	Ascitic	81.1	2.76	185
11	Adenocarcinoma of lung	Pleural	83.3	4.64	
10	Adenocarcinoma of ovary	Ascitic	86.2		174
11	Adenocarcinoma of lung	Pleural	90.9	3.46	
12	Pulmonary tuberculosis	Pleural	94.9	6.43	
13	Adenocarcinoma of lung	Pleural	123.5		181
14	Pulmonary tuberculosis	Pleural	130.3	5.00	245
13	Adenocarcinoma of lung	Pleural	131.5	4.89	
15	Mediastinal lymphosarcoma	Pleural	147.1	4.95	185
13	Adenocarcinoma of lung	Pleural	147.1	5.18	
13	Adenocarcinoma of lung	Pleural	150.0	4.01	
13	Adenocarcinoma of lung	Pleural	151.5	4.64	
13	Adenocarcinoma of lung	Pleural	246.1	4.84	

partition in body fluid often parallels that in the plasma, some notable exceptions may be encountered. Cases 6, 9 and 10

TABLE 3

RELATION BETWEEN THE CHOLESTEROL PARTITION IN PATHOLOGICAL BODY FLUIDS AND IN PLASMA

CASE	DIAGNOSIS	FLUID	FLUID-CHOLESTEROL			PLASMA-CHOLESTEROL		
			Total	Esters	Esters (per cent of total)	Total	Esters	Esters (per cent of total)
			milligrams per cent	*milligrams per cent*		*milligrams per cent*	*milligrams per cent*	
4	Cardiac decompensation	Pleural	29.4	11.8	40.1	110.1	42.0	38.2
13	Adenocarcinoma of lung	Pleural	123.5	85.2	69.0	181.2	101.9	56.2
14	Pulmonary tuberculosis	Pleural	130.3	75.8	58.2	245.0	129.2	52.7
3	Toxic cirrhosis of liver (CCl_4 poisoning)	Ascitic	22.3	13.4	60.1	195.0	104.0	53.3
10	Adenocarcinoma of ovary	Ascitic	86.2	60.4	70.1	173.6	76.2	43.9
9	Primary carcinoma of gall bladder with omental metastases	Ascitic	56.8	41.9	73.8	190.0	37.0	19.5
6	Cardiac decompensation	Pleural	35.8	11.1	31.0	211.0	147.6	70.0

TABLE 4

INDICATING THE VARIATIONS IN FREE AND ESTER CHOLESTEROL IN THE PLEURAL EXUDATES OF A CASE OF CARCINOMA OF THE LUNG AND THE EXTENT OF LOSS OF TOTAL CHOLESTEROL IN SUCH FLUIDS

Case 13, female, aged 35. Diagnosis—adenocarcinoma of lung

DATE OF THORACENTESIS	VOLUME FLUID REMOVED	FLUID-CHOLESTEROL			
		Total	Esters	Esters (per cent of total)	Total in fluid
	c.c.	*milligrams per 100 cc.*	*milligrams per 100 cc.*		*grams*
8/30/33	800	124.0	56.4	45.5	0.99
9/ 5/33	860	113.7	44.7	39.3	0.98
9/ 9/33	1500	123.5	85.2	69.0	1.85
9/13/33	900	125.0	56.8	45.4	1.13
9/20/33	650	147.0	66.4	45.1	0.96
9/27/33	1000	150.0	105.6	70.4	1.50
10/ 4/33	1000	246.1	97.4	39.6	2.46
10/ 9/33	650	151.5	87.2	57.6	0.99
10/14/33	900	131.5	42.2	32.1	1.18
Total.....	8260				12.04

Average plasma cholesterol: 180 mgs. per 100 cc.; calculated plasma volume: 2400 cc.; total cholesterol in plasma: 4.32 grams.

show to what extent the per cent of ester of the total cholesterol in pathological body fluids may deviate from that in the blood plasma.

Table 4 shows the results obtained in a case of adenocarcinoma of the lung. In this patient, a woman of thirty-five years of age, nine thoracenteses were done during a period of one and one-half months. A total volume of 8260 cc. of pleural fluid was removed containing 12.04 grams of cholesterol. This is an exceedingly large amount of cholesterol when one considers that the total blood plasma of this patient contained on the average only 4.3 grams of this sterol. The significance of this finding will be discussed later.

DISCUSSION

In the twenty-one cases reported by Man and Peters and in the thirteen cited in this paper (table 2), the concentration of cholesterol in the blood invariably exceeded that in the effusate. Exceptions, however, may occur as recently pointed out by Stein.[12] He reported a case of so-called "cholesterol-thorax" in which the pleural fluid contained 2353 mgs. of cholesterol per 100 cc. whereas the whole blood showed a concentration of only 74 mgs. per 100 cc. Stein was able to find twenty-one cases of this type in the literature, a condition which is generally associated with tuberculosis. It is conceivable that repeated effusions into the pleural cavity and the inspissation of these exudates by the reabsorption of the fluid elements (cholesterol probably fails to be reabsorbed because it exists in such fluids as large molecular aggregates[3]) eventually gives rise to a concentration of fluid cholesterol exceeding that of the blood.

The results presented in this paper on a patient with adenocarcinoma of the lung (see table 4) are interesting; this person lost 12 grams of cholesterol in the pleural exudates during a period of one and one-half months, a quantity of cholesterol approximately three times that present, on the average, in the total blood plasma of the patient at any one time. Since the total cholesterol content of the plasma showed no significant diminution during the period of study, it follows that the fluid cholesterol probably

originated from one or more of several sources, namely, (1) synthesized cholesterol, (2) dietary sterols, (3) tissue cell breakdown (excluded since this patient manifested no loss in weight or clinical signs of cachexia), (4) cholesterol stores (excluded because of lack of proof that such stores exist in the animal organism*). In recent years, evidence has accumulated to the effect that cholesterol can be synthesized and destroyed in the animal body. Schoenheimer and Breusch[11] recently reviewed the literature on this subject and also reported the results of experiments on mice which prove that cholesterol synthesis and destruction continually take place in the animal organism. Schoenheimer[10] also established the probability of cholesterol destruction in a patient with hypercholesterolemia. Again, the dietary sterols may have been a source for part or all of the cholesterol in the pleural exudates; the cholesterol intake, calculated from the compilation of Twiss and Greene,[13] varied between 0.7 and 1.1 grams per day.† In normal subjects, ingesting this amount of sterol per day, cholesterol balance is probably maintained by variations in the amount of this sterol destroyed, excreted or synthesized. These variables must be considerably altered to maintain sterol balance in patients who lose a large amount of cholesterol through abnormal secretions, as exemplified in the present case and in those patients excreting large quantities of this sterol in the urine (nephrosis).[2] In the case under discussion, it appears probable that the excessive deprivation of body cholesterol was prevented either by the diminished destruction or excretion of ingested sterols or by the increased synthesis of cholesterol. Little else may be safely stated and it remains for future work to determine which of these variables is particularly influenced in morbid states associated with abnormal losses of cholesterol from the organism.

* The liver, adrenals and lungs have been studied by a number of workers under various experimental conditions, but no uniformity in the results has been obtained.

† These figures, undoubtedly, are too high since no differentiation is made between cholesterol and non-absorbable sterols in the food analyses available at present.

CONCLUSIONS

In pathological body fluids, the total cholesterol parallels the total protein content; exudates are characterized by high cholesterol and high protein contents and transudates (including nephrotic edema fluid) by low cholesterol and low protein contents.

The total cholesterol content in pathological effusions bears no direct relation to the total cholesterol content in the plasma. The concentration of this sterol in effusates may approach, but with rare exceptions, does not exceed that in the plasma.

The relation of free to ester cholesterol is alike, at times, in pathological body fluid and in plasma; at other times, the cholesterol partition in effusate and plasma differ markedly.

A serial study of the pleural effusions in a patient with adenocarcinoma of the lung indicates to what extent the human organism can be deprived of cholesterol by abnormal secretions; this patient lost more than 12 grams of cholesterol in eight liters of pleural fluid. Some theoretical considerations concerning the deprivation of body cholesterol are discussed.

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PATHOLOGICAL CHANGES RESULTING FROM ACCURATELY CONTROLLED ARTIFICIAL FEVER*

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The rapidly increasing employment of artificial fever, induced by whatever means, has made a recognized demand for knowledge of the physiological and pathological phenomena accompanying and following it. This is attested by a voluminous literature, most of which has accumulated during the past five years. It would seem, however, that the physiological group of phenomena has been more exhaustively studied than the pathological ones, especially since occasional deaths, attendant upon artificially induced fever, have occurred.

While the reactions which are commonly designated physiological, particularly the chemical alterations in the body fluids, may so far exceed usual limits as to be deemed pathological, we are concerned here primarily with histological changes in the tissues and functional manifestations which may be attributed to those changes. Of these functional manifestations, the blood pressure has been of chief interest. The physiological reactions, essentially identical whether fever be induced by hot baths, heated cabinets, diathermy, or radiothermy, have been summarized by Neymann and Osborne[10], by Bierman[3], and, most recently, by Hench, Slocumb and Popp[7].

Injury from exposure to heat is by no means new. Osler[11] gives to heat stroke the distinction of being the oldest known disease, although its nature was long obscured by superstition. Skin burns must have been familiar to the earliest users of fire. Febrile reaction in infections and in other disease states was

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feared for ages and, even though there has been an increasingly friendly attitude toward fever on the part of physicians since Welch[14] delivered the Cartwright Lectures in 1888,* its potential dangers are still sufficiently obvious. While the kinship of the pathological findings in burns to those in heat stroke and excessive hyperpyrexia is not so clear, the essential identity of the two latter states seems probable.

The early experimental work on heat stroke has been reviewed by Hall and Wakefield[6]. The more recent contributions to the pathology of heat stroke and of excessive hyperpyrexia, can be summarized as follows:

Hall and Wakefield studied dogs placed entirely within a heated and humidified chamber with a dry bulb temperature ranging between 131°F. and 141°F. and wet bulb, between 95°F. and 115°F, for periods of from 20 to 75 minutes. Rectal temperatures of 106°F. to 113.4°F. were produced. Necropsies revealed left ventricles in systole; generalized venous congestion, most marked in the mucous membranes, lungs and liver; blanched and rigidly contracted intestines; dilated stomachs; cloudly swelling of the kidneys; contracted bladders, and petechial hemorrhages. Microscopically there were consistently cellular degenerative changes of varying degree, most prominent in lungs, kidneys, liver intestines, thyroid and brain, and acute passive congestion in all tissues.

Jacobsen and Hosoi[8] elevated the temperature of dogs by means of radiothermy over periods of 37 minutes to 30 hours, 20 minutes, the longest single treatment covering 12 hours. Maximum temperatures of 107.5 to 112.4°F. were attained. Most of the animals died promptly but five were killed after periods of 1 hour to 8 days. These authors summarize their detailed account of morphologic changes as

> congestion of the organs, peripheral hyperaemia, cloudy swelling, fatty degeneration, dehydration, glycogen depletion, focal hemorrhages, especially in the

* It should be noted that Dr. Welch, in these lectures, warned against transferring the results of experiments in heat dyspnea in animals directly to man because in animals respiration has a far more important influence on temperature regulation than in man—a dog pants, a human sweats.

gastro-intestinal tract, epithelial hyperplasia in the parenchymatous organs, stimulation of the bone marrow, and, following prolonged periods of heating, degenerative lesions in the male germinal epithelium.

Rats and guinea pigs, similarly treated, showed similar histologic changes.

Baldwin and Nelson[2] exposed rats to high frequency currents (5–6 million cycles per second) for from 4 to 30 minutes, producing rectal temperatures as high as 113°F. Necropsy findings included a heart usually in diastole; incipient coagulation necrosis of periphery of lungs, heart and liver; a spleen choked with degenerating erythrocytes; necrosis of the periphery of liver lobules; extravasations of blood in kidney with dilated glomeruli; capillaries filled with blood; altered red blood cells, fibrin and cell detritus in Bowman's capsule; and degeneration and exfoliation of the epithelium of the small intestine below the ampulla of Vater, accompanied by leucocytic infiltration.

Baldwin and Dondale[1] repeated the experiments just described, with particular attention to the epithelium of the intestinal tract. Regeneration was found to begin soon after the last treatment and to be complete in most instances by the fifth or seventh day. Occasionally the repair process was delayed over as much as two months.

Schereschewsky[12] concluded, after exposing mice to currents of very high frequency (135–8 million cycles per second), that there was a selective action on tissues varying with frequency. The same author[13], using the same type of current with a frequency of 66–68 million cycles per second, caused softening and necrosis in transplanted carcinomata in mice and attributed these results to some specific activity of that wave length. The tumor necrosis is generally thought to be due probably to heat.

Mortimer[9] heated dogs with both radiothermy and diathermy, as well as rats with the latter, found similar histologic changes after both, and noted hyperaemia and hemorrhages.

Our own experience includes studies of two clinical cases coming to necropsy after fever therapy and of twenty dogs (table 1), eighteen of which were subjected to fever of various durations and

degrees and two of which were used as controls, being given excessive amounts of sodium amytal, a sedative used in all but two of the other experiments and in the human cases.

HUMAN CASES

Case 1—E. P., a white female 20 years of age was admitted June 23, 1934 with complaint of headaches and right lower quadrant pain. She stated that she had always had these bad headaches and that the present attack began five days ago. She had been nauseated but had not vomited. Her pulse was 112; respiration, 24; temperature 102, and blood pressure 110/68. Some colon tenderness was detected; uterus enlarged and retroverted; adnexa enlarged and tender. Leukocytes, 14,750; polymorphonuclears, 73 per cent; small lymphocytes 21 per cent; large lymphocytes 6 per cent. Friedman test positive. A diagnosis of pregnancy with acute salpingo-oophoritis was made.

The patient was given three treatments of 5 hours each in the Kettering hypertherm with temperatures ranging from 103 to 106.6. Sodium amytal, grs. xii, was used as sedative in the first treatment and grs. ix in the other treatments. There were seven day intervals between the treatments. The patient stood the treatments well and the tenderness and induration in the adnexa cleared. However, the temperature continued and the uterus became larger. Two days after the last treatment the patient became very irritable and had a "twitching spell." A similar period of generalized tremor was noted seven days after the last treatment. At this time she had behavior denoting an acute anxiety state and took fluids and food poorly. Three days later, or ten days after the last treatment, the patient was observed in a generalized convulsion lasting one and a half hours after which she lapsed into a deep coma with death on the eleventh day.

Necropsy

Body: Young adult female; lips and mucous membranes cyanotic; considerable postmortem lividity of back: panniculus 2.5 cm. *Abdominal cavity:* Peritoneal surfaces smooth but dry; uterus enlarged, measuring 8 x 10 cm.; spleen and liver usual size. *Thorax:* No adhesions, no fluid; pericardial sac, 10–15 cc. clear fluid. *Heart:* 220 grams; valves intact throughout. *Lungs:* Left 350 grams: posterior portion of lower lobe is dark red in color, congested and edematous. Right lung, 400 grams: lower lobe dark red in color and increased in density; on section shows much congestion and edema. *Spleen:* 60 grams; pulp soft. *Liver:* 1060 grams; capsule smooth; section shows usual architecture. *Stomach:* Usual size and color; mucosa intact. *Pancreas:* Reddish-yellow; normally lobulated. *Adrenals:* Usual size and color. *Kidneys:* Left 140 grams; right 140 grams: on section dark red in color, especially the medulla. *Intestines:* Upper portion of jejunum shows mucosa congested and hemorrhagic in some areas. *Pelvic organs:* Uterus filled with blood clot which reveals 3 months fetus and placenta on section; tubes and ovaries appear

TABLE 1

ANIMAL EXPERIMENTS

DOG	DATE	WEIGHT BEFORE TREATMENT	WEIGHT LOSS	FLUID INTAKE	DURATION TREATMENT	MAXIMUM TEMPERATURE	BLOOD PRESSURE[1]	CLINICAL NOTES	MEDICATION		GROSS PATHOLOGY	SURVIVAL
									Sodium amytal	Morphine		
	1935	*kgm.*	*grams*	*cc.*	*hours*	*°F.*			*mgm. per kgm.*	*mgm.*		*hours*
A	2–15	14.73		qs.	5	108.2	Before: 120/90 After: 70/0 17 hrs.: Unob. 21 hrs.: 96/70	Weak but alert	25 35	32	Congestion of spleen and peritoneal and meningeal surfaces	22[1]
B	2–15	8.75		qs.	5	109.4	Before: 138/100 After: 116/90 17 hrs.: Unob. 21 hrs.: 100/68	Diarrhea and marked prostration	25	32	Cortical hemorrhages, adrenals. Infarcts, both lungs	22[1]
C	2–27	11.66		qs.	5⅔	109.6	Before: 160/100 After: Unob.	No recovery from initial shock	28 18		Generalized congestion	1[3]
D	2–27	13.48		qs.	5⅔	109.4	Before: 200/130 After: 110/80 5 hrs.: 120/90 14 hrs.: Onob.	Reacted satisfactorily. Moribund next noon	28		Hemorrhagic consolidation right lung	18[3]
E	3–13	10.45		qs.	6	107.2	Before: 140/100 After: 130/80 2 hrs.: Unob. 15 hrs.: 130/80	Profound delayed shock Recovery after glucose-saline	25	128	Gross findings not remarkable	40[1]
F	3–13	11.21		qs.	6	108.0	Before: 160/120 After: 126/90 2 hrs.: Unob. 39 hrs.: 190/150	Profound delayed shock Recovery after glucose-saline	25	96	Gross findings not remarkable	40[1]

G	3–20	10.80		qs.	6	108.0	Before: 160/100 After: 132/110 3 hrs.: 30/0 16 hrs.: 176/?	Shock. Bloody diarrhea Cough	25	64	Consolidation, right middle lobe. Ulcer, intestine	65[1]
H	3–20	11.13		qs.	6	108.2	Before: 170/110 After: 148/110 1 hr.: 164/124 3 hrs.: 152–108	Condition apparently satisfactory. Dead next noon	23	128	Massive hemorrhagic consolidation, both lungs	14[3]
I	3–23	12.84		qs.	5	108.8	Before: 158/110 After: 116/90	No ill effects	25 8	64		
	3–25			qs.	6	108.3	Before: 164/116 After: Unob. 6 hrs.: Unob. 15 hrs.: 110/74	Weakness and ataxia, after recovery from shock	25	32	Cortical hemorrhages, right adrenal. Hemorrhage intestinal wall	16[1]
J	4–25	22.14		qs.	0	102.0	Before:[5] 184/120 Lowest: 140/100	Trembling, weakness in hind quarters and ataxia	35 25[6]		Marked congestion, liver and spleen	25[1]
K	4–25	11.66			0	104.3	Before:[5] 158/110 Lowest: 110/78	Ataxic and restless	35 20[6]		Marked congestion, liver and spleen	24[1]
L	5– 8	7.44	90	400	7	107.8	Before: 110/80 After: Unob. 5/9: Unob.	Bloody diarrhea	25	48		
	5–10			200	6	107.6	After: Unob. 5/11: 118/90 5/12: Unob. 5/13: 110/?	Bloody diarrhea and vaginal bleeding	25	64	Hemorrhage around brain stem	72[2]

Unob.—Unobservable.
[1] Killed with Magnesium sulphate.
[2] Killed with chloroform.
[3] Died.
[4] In relation to treatment.
[5] Refers to administration of sodium amytal.
[6] After 5 hours.
Necropsies were all performed immediately after death except in D and H which were examined after about 2 hours.

TABLE 1—*Concluded*

DOG	DATE	WEIGHT BEFORE TREATMENT	WEIGHT LOSS	FLUID INTAKE	DURATION TREATMENT	MAXIMUM TEMPERATURE	BLOOD PRESSURE[4]	CLINICAL NOTES	MEDICATION		GROSS PATHOLOGY	SURVIVAL
									Sodium amytal	Morphine		
	1935	*kgm.*	*grams*	*cc.*	*hours*	*°F.*			*mgm. per kgm.*	*mgm.*		*hours*
M	5– 8	8.58		600	7	108.2	Before: 160/110 After: Unob.	Rectal bleeding Purulent nasal discharge	25	80	Extensive pneumonia, both lungs	$\frac{1}{12}$[3]
N	5– 8	11.79	60	600	7	108.4	Before: 146/106 After: 114/60 5 hrs.: 130/80 19 hrs.: 130/90	Stood treatment well	25	48		
	5–10			300	6	108.0	After: 102/72 7 hrs.: 112/80 5/13: 132/80	Diarrhea. Cardiac arhythmia	25	32	Blood tinged spinal fluid	72[2]
O	5– 8	8–98	90	600	7	107.0	Before: 144/110	Purulent nasal discharge during treatment	25	92	Extensive pneumonia, both lungs	$\frac{1}{4}$[3]
P	5– 8	11.86	150	600	7	105.0 108.6	Before: 152/110 After: Unob. 4 hrs.: Unob.	Purulent nasal discharge during treatment	25	48	Bilateral pneumonia	4[3]
Q	5–17	13.69	450	200	7	106.2	Before: 130/84 After: Unob. 3 hrs.: 110/88 16 hrs.: Unob.	Superficial burn, hind leg. Pulsus paradoxus. Bloody urine		96	Hemorrhagic pneumonia. Abortion. Blood tinged spinal fluid	16[2]
R	5–17	17.13		450	7	108.8	Before: 190/136 After: 30/0 3 hrs.: 96/70 16 hrs.: 140/20	Ataxic but able to walk		196		

	5–18	18.04	630	250	6	106.4	Before: 140/20 After: 146/68 4 hrs.: 108/50 5/19: 160/84	Stood treatment well Walked promptly but with ataxia		176	Hemorrhagic pneumonia	19[2]
S	5–17	14.14	600	None	6	110.4	Before: 128/80 After: 70/0 4 hrs.: 110/80 17 hrs.: 26/0	Bloody diarrhea. Coma lasting 6 hours	25	64	Generalized congestion	17[2]
T	5–17	10.99	630	None	7	107.2	Before: 154/84 After: 50/10 3 hrs.: Unob. 16 hrs.: 70/36	Diarrhea. Weak and ataxic, but walking after 6 hours	25	64		
	5–18			None	6	108.6	Before: 70/36 After: Unob. 5/19: 96/60	Bloody diarrhea. Coma	25	96	Early pregnancy. Hemorrhagic pneumonia, both lower lobes. Bladder distended	19[2]

normal. *Head:* Calvarium usual thickness; there is some engorgement of meningeal vessels but convolutions are of usual width; on section vessels and basal nuclei are markedly congested with minute hemorrhages here and there.

Microscopic examination: Sections from lungs show the parenchyma of the lower lobes hemorrhagic. The bronchioles are well preserved but the alveoli are filled with erythrocytes. Sections from other portions of the lungs show the alveoli filled with lightly staining fluid or air. Sections from adrenals show marked engorgement of the blood vessels and a marked granular degeneration

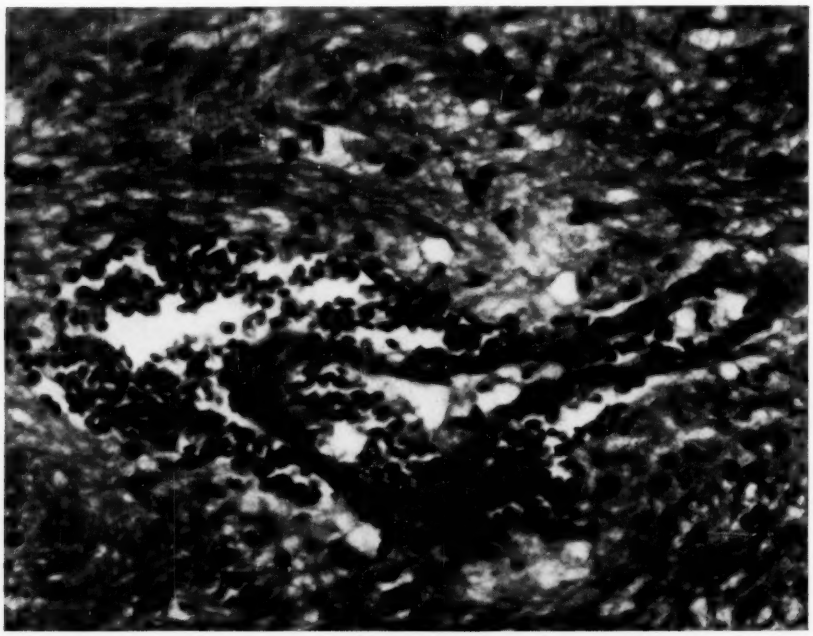

FIG. 1. FIRST HUMAN CASE. Showing cuff-like hemorrhage about medium sized vessel in base of brain (medium power).

of the zona fasciculata. Here the cells seem to fuse, the cytoplasm is pink staining and the nuclei are pyknotic. Sections through the placenta show masses of blood separating the chorionic villi. The centers of many of the villi are homogeneous and pink staining. Sections through the basal nuclei of the brain show marked engorgement of the blood vessels especially those of small caliber. About several of these vessels there is a cuff of erythrocytes infiltrating the surrounding tissue (fig. 1).

Anatomical diagnosis: Pregnancy, about 2 months; degeneration of the chorion with intrauterine hemorrhage; hemorrhagic pneumonia; marked granular

and hyaline degeneration of the zona fasciculata of the adrenals; hemorrhagic encephalitis, ring hemorrhages about vessels in base of brain; acute parenchymatous degeneration of liver and kidneys.

Case 2. G. W., was a male 40 years old. He came to the hospital with the complaint of syphilis. His initial lesion was 20 years ago and he was treated at that time with injections in the hip. For the last six months he had fainting spells and combined treatment with bismuth and potassium iodide was given. Recently he had involuntary clonic movements of right hand and right leg. Physical examination revealed little except Argyl-Robertson pupils and increased reflexes. Both blood and spinal fluid were serologically negative. The impression was that he was suffering from a cerebrospinal type of central nervous system lues with possibility of a tumor causing Jacksonian epilepsy.

Between March and September he was given eleven periods of fever under sodium amytal sedation with the Kettering hypertherm combined with bismarsen. All treatments were tolerated well including the last except for the fact that he could not be aroused. He gradually became more comatose and showed right-sided hemiplegia. Death occurred 20 hours after completion of last treatment.

Necropsy

Body: Middle aged white male; chest flat; abdomen scaphoid; marked cyanosis of lips and mucous membranes. *Abdominal cavity:* Peritoneal surfaces smooth and dry; abdominal organs of usual size. *Thorax:* Pleural cavities show no adhesions, no fluid; pericardial sac 20 cc. clear fluid. *Heart:* 250 grams; valves intact throughout; aorta shows extensive luetic aortitis, characterized by puckered scars and thinned-out areas; orifice of right coronary constricted due to aortitis *Lungs:* Left 670 grams; right 770 grams; lower left lobe edematous and congested with elevated grayish areas of consolidation about bronchioles. *Spleen:* 100 grams. *Liver:* 1500 grams; parenchyma glistening and greasy on section; architecture usual. *Stomach:* Usual size; mucosa markedly congested. *Pancreas:* Reddish-yellow in color; splenic artery thick walled and tortuous. *Adrenals:* Usual size and color. *Kidneys:* Right 150 grams; left 160 grams; cortex smooth, ranges 6–8 mm. on section; parenchyma congested. *Urinary bladder:* Contains 1 liter of clear urine. *Prostate and seminal vesicles:* Usual size. Head: Calvarium thick and opaque; dura adherent to calvarium; there is very little spinal fluid; superficial vessels are dilated and engorged; convolutions unusually broad and flat; there is definite moulding of the cerebellum; on section there is a cyst 2 cm. in diameter surrounded by soft, grayish, tumor tissue at the upper end of the central sulcus on left; lateral ventricles twice their normal size; sections through basal nuclei show vessels congested and small hemorrhages throughout.

Microscopic examination: Sections from the aorta show the elastic fibers of the media broken across by vascular scars in numerous areas. The vessels of

the adventitia are surrounded by cuffs of round and plasma cells. Sections from lungs show the lumina of bronchioles filled with leucocytes and the lining epithelium desquamating. The alveoli surrounding the bronchioles are infiltrated by fluid and polymorphonuclear leucocytes. Sections from adrenals show marked vacuolar and granular degeneration of the zona fasciculata (fig. 2). Sections from brain through wall of cyst and surrounding tumor show the latter composed of closely packed oval or polyhedral cells. The cells have only a moderate amount of stroma and relatively large deeply staining nuclei. Mitotic figures averaging two to the high power field are seen. Sections from the

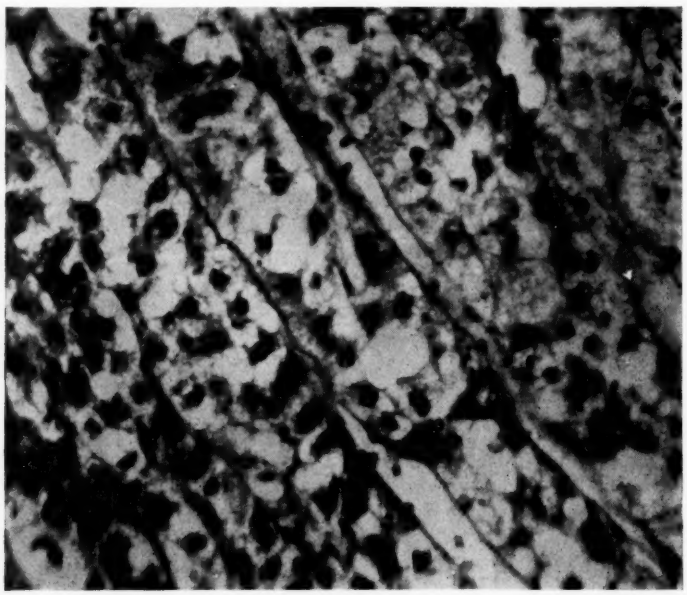

FIG. 2. SECOND HUMAN CASE. Showing marked vacuolar degeneration in the zona fasciculata of the adrenal.

base of the brain show extensive extravasation of blood about the small vessels extending into the surrounding structures. In some areas there are large collections of red cells. About two of the intermediate sized vessels there is a cuff of plasma and round cells.

Anatomical diagnosis: Syphilitic aortitis; partial occlusion of the orifice of the right coronary artery; glioma of cerebrum with cyst formation; hemorrhagic encephalitis, base of brain; bronchopneumonia with oedema and congestion of lungs; vacuolar and granular degeneration, zona fasciculata of adrenals; acute parenchymatous degeneration of liver and kidneys; chronic catarrhal cholecystitis.

ANIMAL EXPERIMENTS

Methods

Female dogs without regard to size, age, or coat were prepared, in most instances, by the intraperitoneal administration of sodium amytal in doses of 25–28 mgm. / kg. of body weight. Supplementary doses of sodium amytal intravenously or, more often, morphia, hypodermically, were given as required. In two dogs (Q and R), for control purposes, only morphia without sodium amytal was given and, in two more (J and K), sodium amytal was given in exaggerated dosage without application of heat. Further preparation included careful bandaging in multiple layers with wide strips of cotton blanketing, this bandaging serving the double purpose of protecting the skin from burns and restraining the dog if restlessness occurred in spite of sedation. These bandages spared only the head which protruded through an aperture in the side of the Kettering hypertherm. This apparatus, an air-conditioned cabinet which was recently described in detail by Desjardins, Stuhler, and Popp,[5] was adapted for the accommodation of two and, later, five dogs. Fluid, in the form of normal saline, was given throughout the treatment to all dogs except two (S and T). During the earlier experiments, this fluid, placed in the mouth with a syringe, was swallowed by the dog. Later it was administered by stomach tube in amounts of 50–100 cc. at a time. Rectal temperatures were taken at intervals during treatment, the usual interval being fifteen minutes. These were recorded, together with the cabinet temperatures, which ranged from 150° to 175°F. Humidity of the cabinet was maintained at 30–40 per cent.

Blood pressures were taken before exposure to heat and at intervals afterward. A narrow pneumatic cuff, connected with an ordinary mercury sphygmomanometer, was placed around the foreleg above the knee and auscultation done over the shaved area, corresponding to the cubital fossa.

Eight of the fourteen dogs sacrificed were given magnesium sulphate intravenously and six were chloroformed. All of these dogs were examined immediately after death. Of the six animals which died, four were examined immediately after death, one two hours after, and one between one and four hours after. Tissues were fixed in formalin and stained with hematoxylin and eosin. Survival was measured from the time of termination of the exposure and varied from five minutes to seventy-two hours.

Five of the animals, as shown in the accompanying table, were given two episodes of fever, two with an interval of twenty-four hours, two of forty-eight hours, and one of seventy-two hours. Thirteen received only one exposure. Duration of any one episode varied from five to seven hours. Maximum temperatures attained varied from 106.2° to 110.4°F.

GENERAL RESULTS

Observations at Necropsy

On opening the body the muscles were usually dark red in color and, in one instance, there was extensive hemorrhage beneath the rectus sheath. In the thorax, the lungs frequently showed areas of dark red consolidation. In one instance, both lungs were completely consolidated and in another, the entire right lung, but for the most part single lobes or parts of lobes were involved. After 48–72 hours, the consolidated areas were grayish-red in color and, on section, oozed yellowish exudate. In the abdomen, the peritoneal surfaces were uniformly smooth and dry. The liver and spleen were usually enlarged and dark red in color. On opening the head, there was invariably edema and engorgement of the blood vessels of the meninges. Actual, basal, meningeal hemorrhage was seen only once, but the spinal fluid was blood-tinged in four instances. Upon section, marked engorgement of the vessels in the base of the brain was noted with small perivascular hemorrhages.

Microscopic Examinations

Sections from the lungs showed great engorgement of the blood vessels with hemorrhage into the interstitial tissue and alveoli. In areas the alveoli were packed with erythrocytes while in others they were filled with bloody fluid. In later stages there was no extensive infiltration by polymorphonuclear leucocytes.

Sections from spleen show marked engorgement but no necrosis or hemorrhage. Sections from liver show marked engorgement of the sinuses with extensive midzonal necrosis and hemorrhage in a few instances. Sections from small intestine show marked engorgement of the vessels with swelling and desquamation of the lining epithelium. The lumen contained varying amounts of blood. Sections from adrenals showed hyaline, granular and vacuolar degeneration of the zona fasciculata of the cortex quite constantly (fig. 3). In some, hemorrhage in this zone was superimposed. Sections from kidneys showed marked engorgement especially of the glomerular tufts. Blood was found fre-

quently in the capsular space and in the tubules. The tubular epithelium showed vacuolar and granular degeneration, especially in the convoluted portions (fig. 4). Sections from brain showed the blood vessels engorged throughout but especially those of the base. About many of these vessels there were ring hemorrhages. The hemorrhages were for the most part about the smaller thin walled capillaries. The surrounding tissue was widely infiltrated in some areas while in others there were rela-

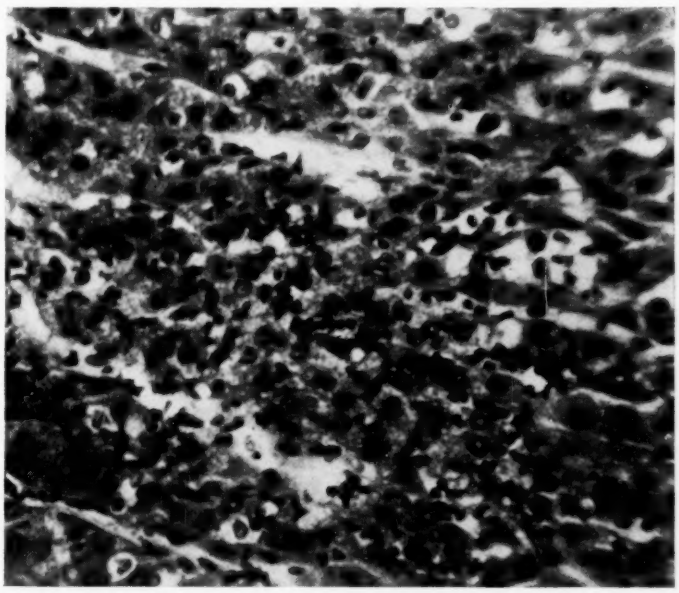

FIG. 3. DOG P. Showing necrosis and hemorrhage in the zona fasciculata of the adrenal (medium power).

tively large solid accumulations around or near the vessel. The ganglion cells frequently showed shrinkage and pyknosis.

REPRESENTATIVE PROTOCOLS

Dog D (2/27/35). A bitch of 13.48 kg. apparently normal, was given sodium amytal, 26 mgm./kg., intraperitoneally and placed in the cabinet at 170°F. As the animal's temperature rose the cabinet temperature was gradually reduced to 155°F. Normal saline was administered freely by mouth throughout the experiment. At the end of one hour rectal temperature was 106.2°F; two hours,

108.2°F; three hours, 107.8°F; four hours, 109.4°F; and five hours, forty minutes, 109°F. At this point the animal was removed from the cabinet. Temperature one hour after removal was 105.3°F and six hours after was 99°F. Blood pressure was 200/130 mm. of mercury before heating and 110/80 thirty minutes after heating. Six hours after heating, the blood pressure had risen to 120/90, but fifteen hours after, it was unobtainable and the animal, previously alert though exhausted, appeared moribund. Respirations were rapid and sighing. Temperature was 98.5°F. Eschatin*, 4 cc., was administered intravenously with 120 cc. of 20 per cent glucose in normal saline. There was prompt but

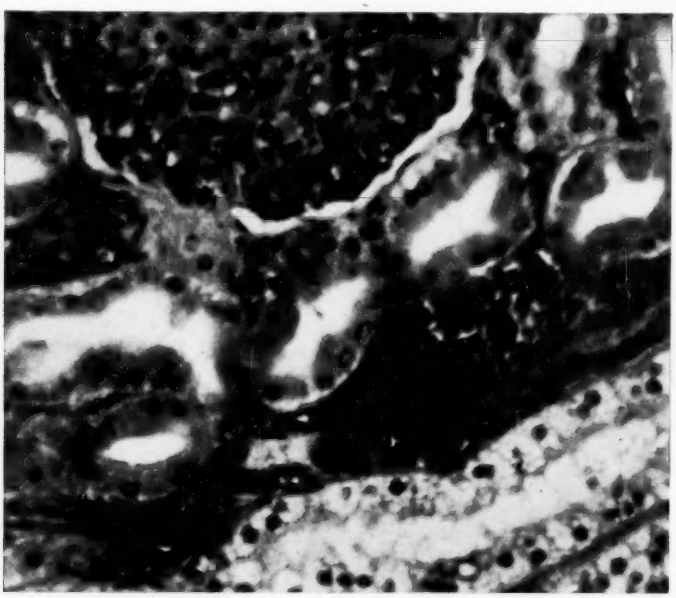

FIG. 4. DOG O. Showing congestion and hemorrhage along with vacuolar degeneration in the tubular epithelium of the kidney (medium power).

transient improvement in alertness and the animal died, eighteen hours after termination of heating. The necropsy, performed two hours after death, revealed the following positive findings: the right lung was entirely and rather firmly consolidated with a dark reddish-brown color and an abundant, foamy, red discharge from the bronchi. The left lung was moderately congested but showed no consolidation. There was a suggestion of minute hemorrhagic areas in the cortex of each adrenal and pallor of the midcortical zone of the kidneys. Moderate congestion of the meninges and surface of brain was present but no gross hemorrhage.

* Furnished by Parke-Davis & Company, Detroit.

Dog R (5/17/35). A bitch, weighing 17.13 kg., apparently normal and with a temperature of 102.1°F., was given morphia, 48 mgms., hypodermically. Seven hours of heating were given, with a maximum temperature of 108.8°F. The cabinet temperature ranged from 150 to 175°F. Morphia, 196 mgms., was given during the course of the treatment in six doses. Blood pressure was 190/136 before start of heating, 30/0 fifteen minutes after termination of exposure, 160/30 after the intravenous administration of 120 cc. of 20 per cent glucose in normal saline, 96/70 three hours after exposure, and 140/20, 16 hours after exposure. Temperature fell to 98.4°F. in three hours and was only 99.8 after sixteen hours. Weight after heating was the same as before. Normal saline, 450 cc., was given by gavage during the course of exposure. The animal survived the episode well and was immediately able to walk, although weak and ataxic. There was no diarrhea.

On the following day with an initial temperature of 99.8°F. and a blood pressure of 140/20, the animal was again placed in the cabinet at a temperature of 154°F. to 175°F. for six hours. Maximum body temperature attained was 106.4°F. Morphia alone, in a total dosage of 176 mgms., was given in five doses. Weight before second exposure was 18.04 kg. Normal saline, 250 cc., was given by gavage. Weight after treatment was 17.41 kg., a loss of 630 grams. Temperature dropped to 99°F. in four hours, and the blood pressure dropped from 146/68, immediately after treatment, to 108/50, after four hours. The dog was able to walk promptly with an ataxic gait. Nineteen hours after the end of the second treatment, temperature was 102.8°F. and blood pressure, 160/80. Chloroform was administered and autopsy performed immediately. There was marked congestion of both lungs with purplish discoloration and early consolidation in both lower lobes. The liver and kidneys exhibited cloudy swelling. The meninges and surface of the brain were congested. No other findings of significance were noted.

Dog T (5/17/35). A bitch, weighing 10.99 kg., apparently normal and with a temperature of 100.8°F., was given sodium amytal, 25 mgms. kg., intraperitoneally. Seven hours of heating were given, with a maximum temperature of 107.2°F. Cabinet temperature ranged from 150 to 175°F. No fluid was given during this episode or the one to follow. Morphia, 64 mgms., in two doses was given hypodermically. Blood pressure was 154/84 before start of heating; 50/10, one hour after termination of exposure; 136/90, after intravenous administration of 150 cc. of 20 per cent glucose in normal saline; unobtainable, three hours after exposure; and 70/36, fifteen hours after exposure. Temperature fell with blood pressure to 99.3°F. and rose, after fifteen hours, to 102°F. Diarrhea had its onset during the heating and continued. Although exhausted and comatose at the end of treatment, the animal was alert after three hours and walking after six. There was, however, marked weakness and ataxia. Weight after heating was 10.36 kg., a loss of 630 grams.

On the following day, with an initial temperature of 102.0°F. and a blood

pressure of 70/36, a second heating of six hours duration was given. The cabinet temperature ranged from 154°F. to 175°F. and the maximum body temperature was 108.6°F. Preliminary sodium amytal, 25 mgms./kg., was given as before and 96 mgms. of morphia, in three doses, were given during heating. At the termination of heating, temperature dropped rapidly to 103.4°F. after forty-five minutes and 99°F. after four hours. Blood pressure, at first unobtainable, rose to 70/0 after intravenous glucose-saline and was unobtainable again after four hours. Bloody diarrhea, coma and prostration were marked. Nineteen hours after end of second treatment, temperature was 101.8 and blood pressure was 96/60. The animal was then chloroformed and necropsy performed immediately. There were hemorrhagic consolidated areas in the lower lobes of both lungs, marked cloudy swelling of liver and kidneys, extreme distention of bladder and congestion of meninges and surface of brain. The spinal fluid was slightly blood-tinged. Early pregnancy was found with no gross hemorrhages into the placentae.

Dog J (4/25/35). A short-haired bitch, weighing 22.14 kg., and apparently normal except that the right kidney had been removed about six months before, was given sodium amytal, 35 mgms./kg., intraperitoneally. Temperature, before administration of sodium amytal, was 102.0°F. Room temperature remained about 72°F. Body temperature fell to 99.4°F. after one hour and fifteen minutes and remained between 99.2°F. and 100.4°F. during the remainder of the experiment. After five hours, a second dose of sodium amytal, 25 mgms./kg., was given intraperitoneally, making a total dosage of 60 mgms. per kg. Blood pressure remained about the same until after the second dose, when it dropped from an initial of 184/120 to 140/100 and remained there. Narcosis was deep after the first dose, accompanied by muscular tremors and shivering, later by athetoid movements of the extremities. After five hours, the animal was still somnolent but easily aroused, was unable to walk, and was still shivering. After the second dose, purposeless movements of the extremities, chiefly the left foreleg, were observed. The following morning the dog was depressed and unable to walk, due to weakness and incoordination in hind quarters. Shivering was still present. After twenty-four hours, standing was possible but uncertain. Magnesium sulfate was injected intravenously and immediate necropsy was performed. The heart was dilated. There was acute congestion of liver and spleen, particularly the latter. No other gross abnormalities were observed except the post-operative absence of the right kidney.

SUMMARY

The pathological changes in both the human cases and the experimental animals were engorgement of the blood vessels, especially the capillaries, hemorrhage and degeneration. The most

vital changes seen were those in the brain amounting to hemorrhagic encephalitis in some instances, those in the lung constituting hemorrhagic pneumonia and those in the adrenal characterized by degeneration in the cortex with hemorrhage. Death occurring during or immediately after treatment was due to vascular collapse. Although the changes described have been noted in animals receiving morphine alone as a sedative, both the human patients and most of the animals received sodium amytal, a drug which in large amounts is known to produce congestion and even capillary damage, especially in the brain.[4,14] Since both the fever therapy and sodium amytal tend to produce marked dilatation and engorgement of blood vessels, it is suggested that the combination should not be used in patients.

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THE VALUE OF THE ONE-HOUR TWO-DOSE GLUCOSE TOLERANCE TEST (EXTON AND ROSE) IN THE EARLY DIAGNOSIS OF DIABETES MELLITUS*

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According to Joslin and Dublin there are 400,000 known diabetics and about half as many undiagnosed cases in the United States. Since the recorded mortality is on the upgrade, in spite of improved methods of treatment, our greatest field of usefulness lies in earlier diagnosis of this disease in order to control it and prevent the distressing complications.

Ordinarily, if the fasting blood sugar level ranges between 80 to 120 mgm. and there is no glycosuria, the possibility of diabetes is too frequently dismissed, even in cases where diabetes might be suspected. There is no doubt that such practice will overlook a certain number of cases of diabetes. With the growing recognition of the close association of familial tendency, obesity, stubborn infections, gall bladder disease and endocrinopathies with diabetes, one should resort to more defining methods in cases which present any such leads. This should surely result in recognizing diabetes before the onset of the classical symptoms.

The alimentary glucose tolerance test furnishes us the best information in this connection. That this test as generally used presents limitations and difficulties of interpretation has been very clearly set down recently by Exton and Rose[1]. These authors have proposed a new alimentary glucose tolerance test calculated to obviate some of the questionable features of the older tests as variously applied. We have performed this new test 150 times and in one third of the cases have checked the new

* Read before the Fourteenth Annual Convention of the American Society of Clinical Pathologists held at Atlantic City, June 7 to 9, 1935.

results by the three hour test previously used in our laboratory. As a result we have come to accept the advantages claimed by Exton and Rose and are using their criteria in diagnosis.

Giving their attention to such important points as the relationship of concentration of glucose solution and absorption rate, shortening the time of test to eliminate emotional factors, and applying Allen's paradoxical law of dextrose, these authors have proposed a "one-hour two-dose glucose tolerance test" as follows:

(1) Collect blood and urine samples, and give first dose of glucose (50 grams in 15 per cent solution) allowing one to two minutes for its ingestion.

(2) Thirty minutes after ingestion of the glucose, collect blood sample and give second dose of glucose (50 grams in 15 per cent solution) allowing one to two minutes for its ingestion.

(3) Thirty minutes after ingestion of the second dose of glucose, collect blood and urine samples.

"The typical criteria of normal responses to the one-hour two-dose test are: (1) a fasting blood sugar within the normal limits of the particular blood sugar method employed; (2) a rise in blood sugar which does not exceed 75 mgms. in the 30 minute sample; (3) the blood sugar in the 60 minute sample is less, the same, or does not exceed the 30 minute sample by more than 5 mgm., and (4) all urine samples are negative to Benedict's test."

"The criteria for determining diabetes in the one-hour two-dose test are a more or less steep rise of not less than 10 mgm. of blood sugar in the 60 minute sample following the second dose of glucose and the relation of blood and urine sugar values to the severity of the disease."

We are of the opinion that the test is based on established physiological principles and believe that because of its relative simplicity, its economy of time both from the standpoint of the patient and the laboratory, its more specific character, its avoidance of equivocal results, it will eventually replace the older methods.

The advantages of the new test in obviating the factor of delayed absorption of concentrated glucose solution, in shortening the time of test and lessening the emotional element, are shown by the following case (fig. 1.):

A young male, 14 years old, came to the hospital complaining of loss of weight, irritability and lassitude. Because of apprehension, aggravated by an animosity toward the resident, the first tolerance test (May, 1933) was misleading in

as much as it presented a diabetic curve. The subsequent follow-up over a two-year period with frequent normal fasting blood sugars, and then an Exton & Rose test (September 1934) (figure 1), showed that this boy was not a diabetic.

Since Pincus and White[4] proved the etiological significance of heredity in diabetes, awakened interest has been shown in determining the sugar tolerance in relatives of diabetics. These

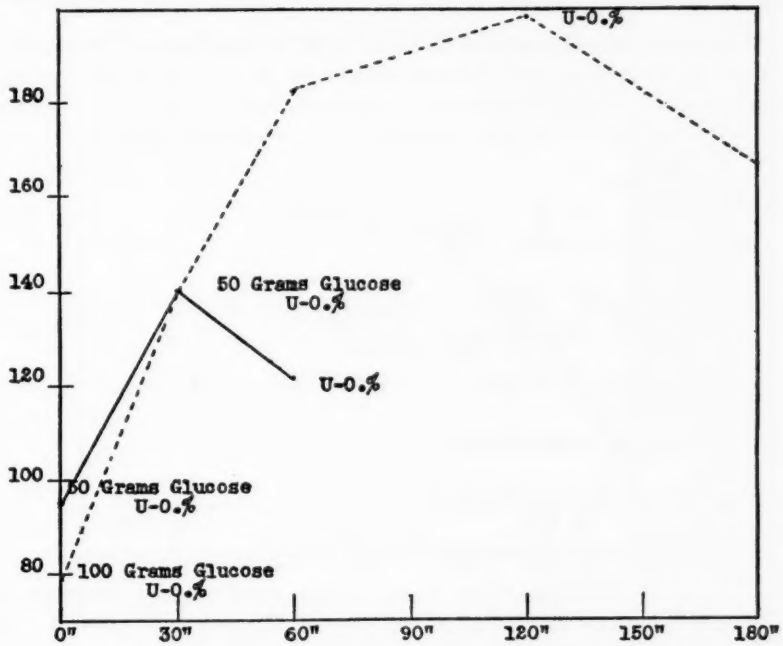

FIG. 1. REPEATED FASTING BLOOD SUGARS NORMAL

The new test, September, 1934, definitely excluded a diabetic tendency; but the older test, May 1933, did not. Patient was refused life insurance June, 1934.

authors[5] found 25 per cent of a group of relatives of diabetics when given sugar tolerance tests, have abnormally high blood sugar values. In applying the new test to four children in a family, both parents of which were diabetic, we found two with typical diabetic curves. One is shown in figure 2.

Joslin[2] has shown that obesity in some degree has been present in about 90 per cent of adult diabetics. With this knowledge it

would seem a reasonable procedure to routinely investigate the overweight individual for diabetes. Because of the frequency with which early diabetics show a blood sugar within normal limits and no glycosuria, we believe it incumbent to apply a stricter test. For this purpose, on account of the reasons given, we have adopted the one-hour two-dose technic. A case in point is the one illustrated in figure 3.

This patient, a white male of 35 years, came for treatment of "nervousness" of three years duration. He weighed 259 pounds, gave a family history of many members of it being overweight, and of two paternal aunts dying of diabetes.

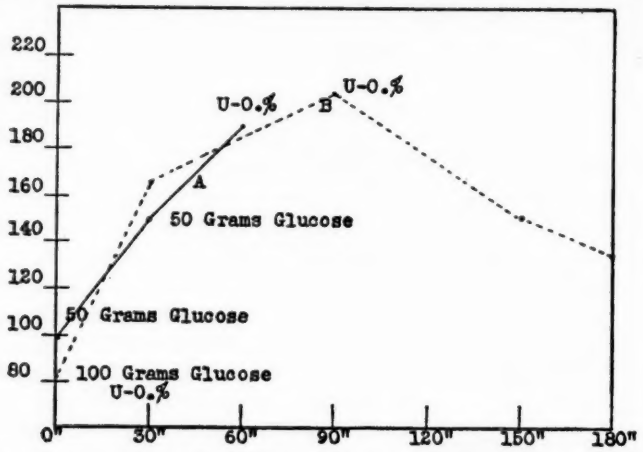

FIG. 2. (A) ILLUSTRATES NEW TEST. CHILD OF DIABETIC MOTHER AND FATHER. ONE OF FOUR CHILDREN, TWO OF WHOM ARE DIABETIC. (B) OLD TEST

According to Joslin[3] "in adults cholelithiasis is probably one of the most important etiological factors in diabetes mellitus." It would seem from this that the surgeon has not fully discharged his duty to the gall bladder patient until in addition to the surgical treatment, he has had an evaluation of the carbohydrate metabolism. Figure 4 shows two interesting curves in a patient whose chronically infected gall bladder was removed in 1922. The first test by the older method gave a doubtful curve. The new test gave a curve which was unquestionable.

The rôle of diabetes in lowering resistance to infections and

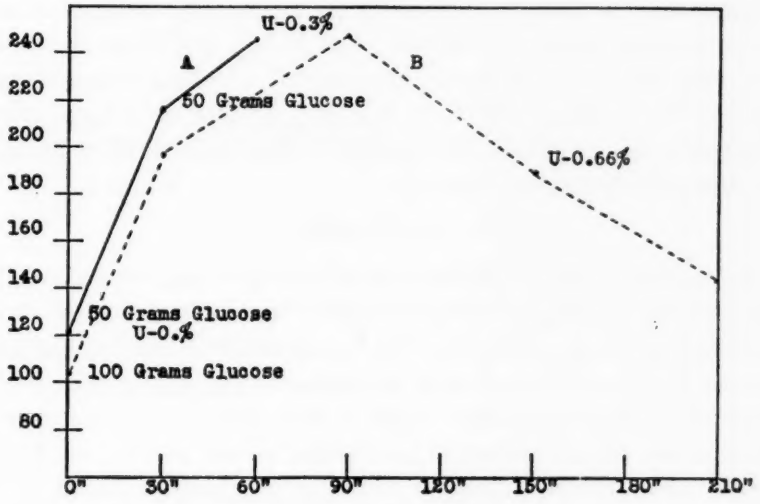

FIG. 3. (A) ILLUSTRATES TYPICAL DIABETIC CURVE. FAMILY HISTORY OF OBESITY AND DIABETES. WEIGHT OF PATIENT 259 LBS. (B), OLD TEST

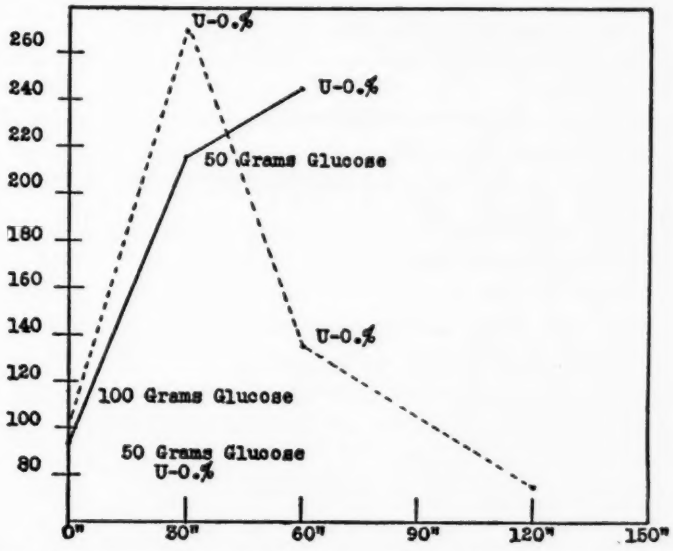

FIG. 4. THE CONTINUED CLIMB OF THE BLOOD SUGAR CURVE AFTER THE SECOND DOSE OF GLUCOSE IS TYPICAL OF DIABETES

The curve of the older test is atypical. Cholecystectomy, 1922.

predisposing to their intractability is so generally recognized as to put one immediately on guard. An Exton and Rose curve was obtained typical for diabetes, in a patient who had a long delayed healing of a burn on the foot, followed by two deep hypodermic abscesses. The fasting blood sugar in this case was 91 mgm. with no sugar in the urine.

CONCLUSION

In presenting our experience with this new test, we realize that our series is not large; but we do believe it sufficient to warrant our acceptance of the claims for the "one-hour two-dose glucose tolerance test." We hope that by doing so wider attention will be directed to its superiority. This in turn should lead to its use in those cases where accurate knowledge of the sugar metabolism would be of value in making an early diagnosis of diabetes; the advantage to the patient no one denies.

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THE ROSE BENGAL LIVER FUNCTION TEST AS ADAPTED TO THE SHEARD-SANFORD PHOTELOMETER*

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Since Delprat[1] in 1923 first introduced the rose bengal test for liver function, he and his associates[2,3,4,6] have accumulated a vast amount of clinical and experimental data which definitely has proved that the dye is removed from the blood stream exclusively by the liver and excreted in the bile and that the dye can be administered safely without any unpleasant reaction or symptoms. The clinical data offered confirms their conclusion that the rate of excretion of the dye is quite parallel to the normal activity of the liver cells. From time to time the technic has been improved, but still the test has not been generally adopted as one would expect, especially when the need for such a test is so great. During the past twenty years many liver function tests have been introduced, including the galactose tolerance test, the bromsulphalein and phenoltetrachlorphthalein dye test, et cetera. The criticism of many of these tests has recently been discussed by Stowe, Delprat, and Weeks[9] and will not be reviewed here except to mention that none of these tests has gained very wide acceptance.

Our earlier work with the various dye tests met with many technical difficulties in estimating colorimetrically the exact concentration of dye present. The difficulty still exists even with the most recent technic published by Stowe and others and may account for the restricted use of this test. In addition, other complications are met, particularly when dealing with samples having a high bile retention in the blood.

The purpose of this report is to record our own experience with

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the rose bengal test, point out some technical difficulties encountered and describe our own technic in performing the test with the Sheard-Sanford[7] photelometer.

TECHNIC

The technic is practically the same as that recently published by Stowe for the colorimetric method. Rose bengal for intravenous use is obtained from Coleman and Bell. A 1 per cent solution is made by dissolving the dye in normal salt solution for a period of three hours shaking at intervals.[8] It is then filtered through Whatman filter paper #2 into a pyrex flask or put into ampules as desired and autoclaved for twenty minutes at ten pounds pressure. Care is taken to keep the dye in a dark place since it has been shown to be photosensitive in direct sunlight. Patients injected with this dye should be protected from the sunlight from two to three hours after the injection and in our experience we have found it necessary to protect patients with obstruction to the common bile duct from sunlight until the dye is all excreted. The equipment consists of three sterile syringes, two of which are filled with 10 cc. of sterile physiologic saline, and the third with 10 cc. of 1 per cent rose bengal solution, a #18 gage needle, a small tube containing no oxalate, and three oxalated bottles numbered 1, 2, and 3. The needle is introduced into one of the cubital veins and 10 cc. of blood are withdrawn. About 3 cc. of the blood are transferred to the test tube and the balance is placed in the oxalated bottle #1 and mixed thoroughly. As this syringe is disconnected from the needle, another containing the 10 cc. of rose bengal solution is attached and the solution injected during a period of thirty seconds which is carefully timed. (In children 5 cc. of the dye are given.) The needle is left in place and 10 cc. of normal saline are slowly injected over a period of exactly two minutes from the time the injection of the dye was begun. At the end of the two minute interval, another 10 cc. of blood are removed to oxalated bottle #2 and mixed. A third syringe is now attached to the needle and 10 cc. of salt solution are gradually injected over a period of exactly six minutes. At the end of eight minutes from the beginning of the dye injection, the last sample of blood is removed and placed in oxalated bottle #3. The patient and the nurse are both informed that the subsequent stool will be rose tinged.

The samples of blood are centrifugalized and 5 cc. of plasma are placed in each of three graduated centrifuge tubes to which are added two volumes of acetone to precipitate the proteins. The tubes are allowed to stand for three minutes or longer in subdued light and then centrifugalized at high speed until a clear supernatant fluid is obtained. We have found that plasmas with low icterus index will have a sparkling rose color from bottles #2 and #3, while those with high icteric values may have no rose color. The supernatant fluid is transferred to centrifuge tubes and five drops of concentrated NaOH are added and mixed well. In view of the fact that NaOH attacks the skin, either clean rubber corks or rubber finger cots are used in shaking these tubes. Shak-

ing in a horizontal plane insures complete mixing of the NaOH which otherwise tends to remain at the bottom of the tubes. The NaOH apparently converts the acidic bilirubin into the water soluble bilirubinate and extracts it from the plasma. The tubes are allowed to stand for fifteen minutes and are centrifugalized for five minutes. If the NaOH at the bottom of the tubes has little or no bilirubin dissolved in it, the rose colored supernatant fluid is ready for colorimetric and photelometric determinations. However, if the NaOH at the bottom of the tubes contains a large amount of bilirubin the tubes are permitted to stand for one hour and again the supernatant fluid is transferred to clean tubes with the addition of another five drops of NaOH. This procedure is repeated until no more yellow color is present in the NaOH that accumulates at the bottom of the tubes, while the supernatant fluid develops a brilliant rose color. We have found that plasma containing a large amount of bilirubin also contains a high fat content and at times this imparts a cloudiness to the solution which can be eliminated by the addition of ether. As the control plasma is treated in the same way as samples #2 and #3 usually it will appear free from bilirubin. Colorimeter readings are made first and then immediately the solutions are placed in the photelometer cups containing the glass adapters as previously described[5] making the reading possible with a small amount of fluid (8 cc.).

In normal patients the colorimeter readings are made with less difficulty than in those with hepatic disease as the character of the color in the two samples is often similar. However, when a large amount of dye has been excreted and the second sample contains very little color it is incomparable with the first rose colored sample. When the bilirubin content is high and the dye excretion is low, the two samples appear to be of the same order of color. However, we find upon attempting to read them in the colorimeter, the first sample is a rose color, and the second has a decided coral hue. Removal of a large amount of bile salts with concentrated NaOH usually helps but does not entirely solve the problem. Because of these difficulties, this procedure also did not meet the requirements of a satisfactory test. With the introduction of the photelometer by Sheard and Sanford, the investigation was renewed and this instrument was used in conjunction with the colorimeter for a comparative study. In using the photelometer we are able to compensate for the high bilirubin content of the plasma by placing the pre-dye plasma-acetone mixture in the control cell and setting the needle at 100 on the galvanometer so that only the rose color is estimated. Readings in normal cases

are made by using water in the control cell and subsequently compared with readings obtained by using the plasma-acetone mixture in the control cell. These readings are practically the same even in plasma originally containing high bilirubin content, since the NaOH extracts proportionally the same amount of bilirubin from each tube. Should the final amount of plasma prove

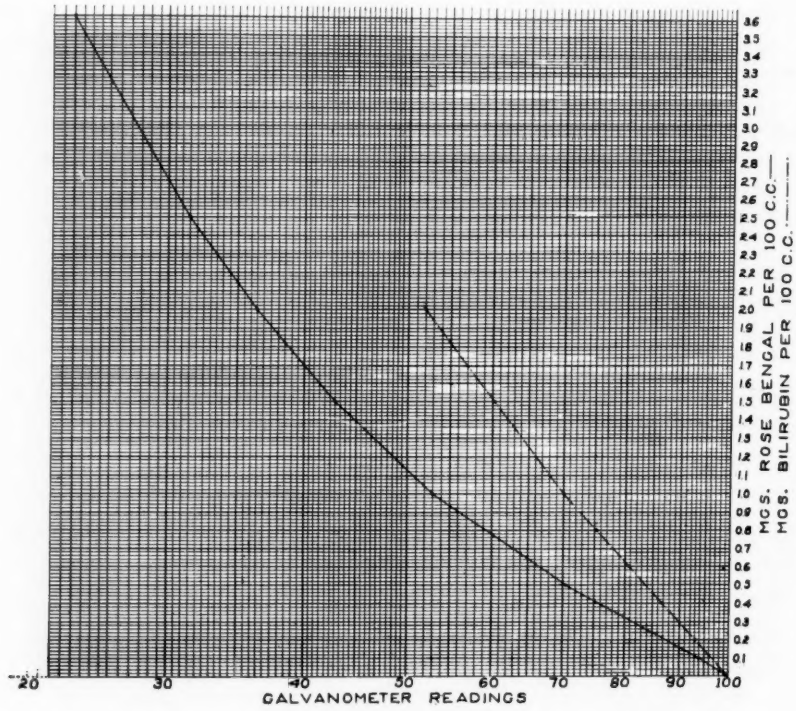

FIG. 1. GRAPH ILLUSTRATING ROSE BENGAL AND BILIRUBIN CURVES

insufficient to fill the photelometer cup, the addition of acetone or ether will be found satisfactory to increase the volume.

In using the Sheard-Sanford Photelometer for the rose bengal test, the following method was used to obtain the standard curve:

1 cc. of the 1 per cent rose bengal solution was diluted to 100 cc. with physiologic saline in a volumetric flask. 1 cc. of this solution contains 0.1 mgm. rose bengal. From this stock preparation 1, 2, 3, 4, 5, 6, and 7 cubic centimeter

TABLE 1

Calibration Table for Rose Bengal Determinations

PHOTELOMETER READINGS	ROSE BENGAL	PHOTELOMETER READINGS	ROSE BENGAL	PHOTELOMETER READINGS	ROSE BENGAL
	mgm.		*mgm.*		*mgm.*
25.0	3.600	50.0	1.139	75.0	0.414
25.5	3.509	50.5	1.116	75.5	0.405
26.0	3.416	51.0	1.092	76.0	0.395
26.5	3.325	51.5	1.069	76.5	0.386
27.0	3.236	52.0	1.046	77.0	0.377
27.5	3.149	52.5	1.023	77.5	0.368
28.0	3.062	53.0	1.000	78.0	0.359
28.5	2.978	53.5	0.983	78.5	0.350
29.0	2.895	54.0	0.967	79.0	0.342
29.5	2.814	54.5	0.951	79.5	0.333
30.0	2.733	55.0	0.934	80.0	0.324
30.5	2.654	55.5	0.918	80.5	0.316
31.0	2.576	56.0	0.903	81.0	0.307
31.5	2.500	56.5	0.887	81.5	0.298
32.0	2.446	57.0	0.872	82.0	0.290
32.5	2.394	57.5	0.857	82.5	0.281
33.0	2.342	58.0	0.842	83.0	0.273
33.5	2.291	58.5	0.826	83.5	0.265
34.0	2.241	59.0	0.812	84.0	0.256
34.5	2.191	59.5	0.797	84.5	0.248
35.0	2.142	60.0	0.782	85.0	0.240
35.5	2.095	60.5	0.768	85.5	0.232
36.0	2.047	61.0	0.753	86.0	0.224
36.5	2.000	61.5	0.739	86.5	0.216
37.0	1.960	62.0	0.725	87.0	0.208
37.5	1.918	62.5	0.712	87.5	0.200
38.0	1.877	63.0	0.697	88.0	0.192
38.5	1.837	63.5	0.683	88.5	0.184
39.0	1.798	64.0	0.669	89.0	0.176
39.5	1.759	64.5	0.656	89.5	0.168
40.0	1.721	65.0	0.642	90.0	0.160
40.5	1.683	65.5	0.629	90.5	0.153
41.0	1.645	66.0	0.616	91.0	0.145
41.5	1.608	66.5	0.602	91.5	0.138
42.0	1.572	67.0	0.589	92.0	0.130
42.5	1.536	67.5	0.576	92.5	0.122
43.0	1.500	68.0	0.563	93.0	0.115
43.5	1.472	68.5	0.550	93.5	0.107
44.0	1.444	69.0	0.538	94.0	0.100
44.5	1.418	69.5	0.525	94.5	0.092
45.0	1.391	70.0	0.513	95.0	0.083
45.5	1.365	70.5	0.500	95.5	0.074
46.0	1.338	71.0	0.490	96.0	0.066

TABLE 1—*Concluded*

PHOTELOMETER READINGS	ROSE BENGAL	PHOTELOMETER READINGS	ROSE BENGAL	PHOTELOMETER READINGS	ROSE BENGAL
	mgm.		*mgm.*		*mgm.*
46.5	1.312	71.5	0.480	96.5	0.058
47.0	1.287	72.0	0.471	97.0	0.049
47.5	1.262	72.5	0.461	97.5	0.041
48.0	1.237	73.0	0.451	98.0	0.033
48.5	1.212	73.5	0.442	98.5	0.024
49.0	1.188	74.0	0.433	99.0	0.016
49.5	1.163	74.5	0.423	99.5	0.008

amounts were pipetted into 100 cc. volumetric flasks and made up to volume with physiologic saline, each of these solutions representing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 milligrams per 100 cc. respectively. These solutions were then carefully read on the galvanometer using the green Wratten filter #74* in the photelometer and distilled water in the control cell. The results were tabulated and the points plotted on the Keuffel and Esser semi-logarithmic paper (1 cycle × 60 divisions) as shown in figure 1. A slope line was drawn to join the seven points. Reading and recording the intermediate points on this line is a laborious process and we found the slide rule to be exceedingly helpful, as adjustments could be made easily where the line was not exactly straight. A convenient and readable table (table 1) was then made. This table is applicable to any photelometer without further standardization in view of the fact that the amount of rose bengal contained in the two samples of blood is relative in proportion.

As stated by Delprat et al. the greatest concentration of dye in the blood is present in about two minutes from the time of injection. A normally functioning liver is capable of removing about 50 per cent of the dye from the blood in six minutes, thus leaving 50 per cent to be excreted later. The normal range of liver function varies from 85 per cent to 115 per cent. The calculations involved are shown in the following example:

If the reading of the first sample is 0.8 mgm. and the reading of the second sample is 0.5 mgm. then the amount excreted is 0.3 mgm. or $\frac{0.3}{0.8}$ mgm. The

* Supplied by Central Scientific Company.

percent of liver function then is 200 times the fraction of dye excreted or the formula $200 \times \frac{(R^1 - R^2)}{R^1}$.

R^1 = reading of the first sample and R^2 = reading of the second sample.

For colorimeter determinations the following formula is applicable:

$$200 - \frac{(200 \times Rs)}{Ru}$$

Rs = reading of the standard and Ru = reading of unknown.

TABLE 2

COMPARATIVE READINGS OF THE PHOTELOMETER AND COLORIMETER

PHOTELOMETER READING		COLORIMETER READING	PHOTELOMETER READING		COLORIMETER READING	PHOTELOMETER READING		COLORIMETER READING
Plasma	Water		Plasma	Water		Plasma	Water	
per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
87	87	85		60	56		82	89
77	77	80		33	32	94	95	100
95	103	104		37	34	64	61	65
94	95	80	33	35	28		54	47
	92	106	111	105	110		57	58
	111	117	97	97	104		38	43
70	71	80	96	96	100		103	83*
95	88	94	99	90	89		18	34*
88	88	80	63	59	53		80	95*
	84	80		105	100		77	67*
	120	114	53	42	50	80	76	64*
	26	31		78	80	25	23	11*
	10	9	65	57	55	80	77	67*
33	31	30		69	67		51	43*
	50	49	89	92	83	64	55	64*

* Approximate colorimeter readings.

In a comparative study covering sixty-one determinations, 13 or 21 per cent could not be read with the colorimeter and in 9 or 14 per cent, the colorimeter readings were regarded as approximate because of variation of color in the two samples. In this group we find the greatest variation between these two instruments as shown in table 2. Notice also the very close agreement between the reading obtained with water and with the plasma-acetone mixture in the central cup. Up to this time the

dye has been injected seventy-nine times. One patient has received the dye eight times; one, five times; three, four times; and three patients three times each. These cases represent a wide range of normal controls and a small group with pathologic conditions involving the biliary tract. In the entire series of injections no complaints were made by any of the patients. However in two instances definite abnormal symptoms were produced and manifested by itching, swelling of face, hands, and feet. Both of these patients had a low liver function and a high bilirubin content in the blood. This occurred when these patients

TABLE 3

SUMMARY OF CLINICAL COURSE OF CASES 1 AND 2

CASE	DATE	LIVER FUNCTION	ICTERUS INDEX	VAN DEN BERGH DIRECT REACTION	CLINICAL NOTES
		per cent	*units*		
1	3- 5-35	57	9	Delayed positive	
	3-21-35	69	4	Negative	
	3-29-35	92	3	Negative	
	4- 6-35	89	3	Negative	
2	2-15-35	22	40	Negative	Skin and sclerae yellow; still nauseated
	3- 1-35	47	25	Negative	Jaundice much improved
	3-16-35	67	17	Negative	No jaundice; patient felt well
	4-20-35	58	5	Negative	No complaints
	6-28-35	98	4	Negative	

were exposed to the sunlight as late as one day after the injection of the dye, thus indicating that patients with low excretory power to the Rose Bengal dye should not be permitted to be exposed to sunlight until one is certain that all of the dye has been excreted. While the clinical interpretation of the cases studied will be considered fully in another communication, the following two case reports will serve to illustrate the accuracy of the test.

CASE REPORTS

(1) A male, 58 years of age, was admitted to the hospital for exploratory laparotomy. A gastric lesion was suspected but none was found. The liver

was small and the surface rough. Small section of liver was removed for examination. Two days following the operation (3/15/35) the rose bengal liver function test was 57 per cent. The icterus index was 9 units and the direct van den Bergh gave a delayed positive reaction. The patient was placed on high carbohydrate diet. The post-operative course was uneventful and the subsequent liver function tests are shown in table 3. The microscopic exam-

FIG. 2. CASE 1. ILLUSTRATING FATTY INFILTRATION WITH MARKED PERIPORTAL LYMPHOCYTIC AND POLYNUCLEAR INFILTRATION
(Hematoxylin and eosin stain. × 250)

ination of the liver revealed extensive fatty infiltration with marked periportal fibrosis and round cell infiltration. Some of the liver cells in the midzone of the lobule contain bile pigment and showing fatty degeneration (fig. 2).

(2) A female, 22 years of age, whose present illness began December 1934 with an upper respiratory infection, in the early part of January 1935 noted a gradual onset of jaundice associated with nausea and occasional vomiting.

The icterus progressively increased up to a serum value 120 units. Patient returned February 15, 1935 and at this time the icterus was mild, the patient clinically much improved, with no complaints. The rose bengal liver function was 22 per cent, the icterus index 40 units, and the van den Bergh direct reaction was negative. The patient was ambulatory and was kept under observation. The liver function tests and clinical course are summarized in table 3.

The value of the test is well illustrated in the first case. This patient presented no clinical symptoms and an exploratory laparotomy revealed an early cirrhosis of the liver. The liver function at this time was low. The history later revealed that this patient for years had consumed a large amount of beer, but with proper dietary management the liver function gradually improved up to a certain level and then remained stationary. It is interesting to note that the icterus index was within normal limits and yet the van den Bergh test gave a delayed positive reaction. The second patient is also very interesting in that the liver function did not improve to the same degree as the clinical examination would indicate. Thus suggesting that the Rose Bengal test for liver function is actually a better indicator of the physio-pathologic state of the liver than the clinical history and physical examination.

CONCLUSIONS

An improved technic for the Rose Bengal liver function test has been presented together with some of the difficulties encountered. The present evidence sustains the conclusion of the originators of the test, that for clinical purposes it is accurate and safe. The technic is simple and the dye inexpensive.

The authors are indebted to the Medical Staff of the St. Joseph and Epworth Hospitals of South Bend, Indiana and St. Joseph Hospital, Mishawaka, Indiana for their cooperation and encouragement in obtaining these data.

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A METHOD FOR THE QUANTITATIVE EXAMINATION OF CONJUNCTIVAL FLUID IN PREPARATION FOR INTRA-OCULAR OPERATIONS*

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Addressing the 1932 meeting of the Opthalmological Society of the United Kingdom in a symposium on senile cataract, Dr. A. J. Ballantyne[1], of Glasgow, Scotland, said:

> Antisepsis and asepsis have not achieved the same triumphs in ophthalmology as in general surgery, and we cannot yet discount the danger of infection in intra-ocular operations; indeed wound infections and infections of the iris and ciliary body are still among the most formidable of the post-operative complications which we have to face, and pre-operative care and preparatory treatment of the patient are largely directed to the elimination of these dangers.

It is apparent from modern literature dealing with ophthalmologic surgery that beyond a mildly antiseptic treatment and mechanical cleansing immediately before entering the eyeball the surgeon depends upon the relative freedom of the conjunctival mucosa from pathogenic bacteria. The entry of frankly pathogenic bacteria in sufficient numbers and their implantation on traumatized tissue may result in a rapid primary pyogenic infection with loss of the eye or a secondary non-pyogenic infection that may *greatly impair the operative result.*

An eye presenting a clinical conjunctivitis is rejected for operation until clinical normality is manifest after more or less prolonged treatment with a great variety of drugs. With the rare clinical and operative ability of Professor Fuchs of Vienna, he is credited by Dr. W. A. Fisher[2] of Chicago as losing about 0.5 per cent of cases from infection in an annual series of approximately

* Read before the Fourteenth Annual Convention of the American Society of Clinical Pathologists held at Atlantic City, June 7 to 9, 1935.

500 cases. In less skillful practice the incidence of loss must be much higher. I have not been able to find actual figures on this point.

The frequency of intra-ocular operations is considerably more than one would imagine. Counting those of most risk from operative entry into the eyeball, such as trephining, iridectomy, iridodesis, discission and cataract removal, a total of 536 operations were performed in the New Orleans Charity Hospital in 1934. The private cases operated upon in New Orleans will slightly exceed this number, making at least 1000 such operations annually in one city of a half million population.

In practically all of the late English and American books and papers by opthalmologists of note it is recommended that "smears and/or cultures" be made; and, as most recently stated by Dr. C. A. Clapp[2],

> If the culture from the conjunctival sac shows the presence of streptococci, or particularly of pneumococci, the operation should be postponed until cultures are negative for these organisms.

Staphylococcus aureus and *citreus* are also included as operative deterrents by several authors.

Ballantyne mentioned Elsching's method of instilling culture media into the eye, pipetting this out and plating it on media in a Petri dish, but such a method is obviously qualitative only.

Professor Lundsgaard[5], urged the use of a cotton swab to scrape off the surface epithelium and also insisted upon the use of blood agar plates. He discredited, but gave no reference to, a method by Wissman of inserting a sterile silk thread into the conjunctival sac where it is allowed to soak for a few minutes before removing and culturing directly.

As a clinical pathologist and consultant with the ophthalmologist it has seemed that I should bear the responsibility of absolutely determining the relative freedom of the conjunctival sac from pathogenic bacteria. I have searched the literature for some quantitative method for evaluating the bacteriologic status of this sac without avail. The use of platinum loops or spatules or cotton swabs or pieces of thread is obviously uncertain in the

delivery of even a fraction of a drop of conjunctival fluid to the culture medium.

Evidence of the presence of pyogenic infection of the mucosa of the conjunctival sac may also be shown by an increase in the number of leukocytes. A total leukocyte count and a quantiative as well as a qualitative bacterial determination should also be much more accurate in determining sub-clinical infections than either clinical observation or the above mentioned methods.

METHOD OF CULTURE

A capillary pipette 1 mm. outside diameter made from 4 or 5 mm. glass tubing was found to give an average column height of 10 cm. with 0.02 cc. of fluid. By bending the last few centimeters at right angles (fig. 1), rounding the tip in a small flame, marking a one-half centimeter distance mark from the tip, and the use of rubber tubing equipped with throttle pipette and mouth piece for suction, an instrument was devised for obtaining approximately 2 cu. mm. of fluid for inoculation of media or for obtaining a somewhat larger quantity of fluid to mount on a hemacytometer for cell counts. The butt end of the pipette was plugged with cotton and it was dry sterilized.

In practice the lacrimal sacs are evacuated into the conjunctival sac with gentle pressure. Massage over the eyelids serves to average the fluid contents and tends to desquamate loosened epithelial cells. The lower lid is retracted and the capillary touched to the lower portion of the fold next to the sclera (fig. 1) and filled to the mark with fluid. This is ejected onto the surface of a blood agar plate and spread about by the bent end of the pipette. Another quantity of fluid is obtained and mounted directly on a Bass-Johns[4] hemacytometer which is so constructed that any portion or an entire cubic millimeter of fluid may be counted directly without using a factor. Dilution of the fluid by increased lacrimation does not occur with gentle handling of the pipette.

By these quantitative means it was first determined that the conjunctival fluid of fifty-four normal young adults contained from 1 to 250 leukocytes per cu. mm., with an average of thirty-

five cells. Cultures were not made on many members of this group. *Bacillus xerosis* (diphtheroids) and a few *Neisseria catarrhalis* or *Staphylococcus albus* were the usual findings. Several cases of mild clinical conjunctivitis showed counts of well over 1000 leukocytes with numerous colonies of bacteria. One hundred and fifty-five combined leukocyte and bacterial counts were

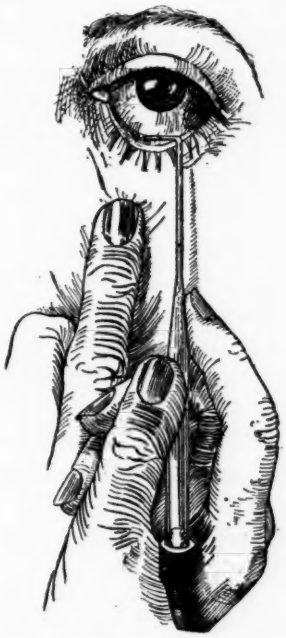

FIG. I. SPECIAL PIPETTE FOR TAKING CULTURES

With finger partially everting the lower lid and capillary pipette held in other hand and steadied on finger tips the desired quantity of fluid is drawn up into tip of pipette.

made on seventy-seven cases selected for iridectomy, cataract or trephine operations in private practice. Of this group sixty-eight were operated upon without subsequent primary or secondary infection. An analysis of the findings necessitated a division of the patients into three groups. One of these received no local treatment for several days at least before the final examination. The second group received a great variety of local antiseptic

medication. The third group were not followed to operation or were rejected for other reasons than the laboratory findings.

Group 1. Of thirty-five patients who had not been subjected to local treatment the leukocyte count varied from 1 to 1105, with an average count of 107 per cu. mm. of fluid. The bacteria averaged 14 colonies of *S. albus*, 0.6 colony, *S. aureus* and 20 colonies of *B. xerosis*.

Group 2. Of thirty-three patients who had received various types and frequencies of antiseptic treatment prior to the last examination the leukocytes varied from 6 to 5,100 with an average of 677 leukocytes per cu. mm. The bacterial counts in this group gave an average of 5 colonies of *S. albus*, 0.8 colony of *S. aureus*, 0.09 colony pneumococci, 0.06 colony *N. catarrhalis* and 12 colonies *B. xerosis* per cu. mm. of fluid.

Group 3. The remaining nine patients were not operated upon. Two are still being delayed on account of leukocyte counts well up into the thousands with confluent growths of staphylococci. Seven others were unsuitable for other reasons.

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THE DETERMINATION OF PROTEIN IN CEREBROSPINAL FLUID

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There are three types of methods in common use for determining the amount of protein in cerebrospinal fluid. These are: (1) methods which depend on precipitation with some one of the many protein precipitants, and comparison of the turbidity produced with that given by a known amount of protein treated in a similar manner, (2) colorimetric methods in which the amount of some amino acid is determined and the protein is calculated from the results obtained, (3) methods depending on the precipitation of protein and the subsequent determination of the nitrogen in the precipitate.

Of these three types of methods the one described first is the most convenient, and, in our opinion, the least exact. Two reasons for this lack of accuracy may be given: (1) many persons find it difficult to compare turbidities accurately, even with the aid of such instruments as the nephelometer and the turbidometer; (2) the degree of turbidity produced depends not only on the amount of precipitatable material present, but also upon the composition of the solvent, such as the amount of salts and other soluble material which it contains. Very slight variations in these compounds seem sometimes to affect significantly the readings given by precipitated protein.

Estimation of the protein by precipitation with some convenient reagent, determination of the amount of some one of the amino acids contained in the precipitate, and calculation of the protein from the value found is more satisfactory. Although such methods seem to carry a rather high error[3], it is probably not often great enough to affect the clinical interpretation of the results. A theoretical objection to such methods is that it is not certain that

all proteins contain the same amount of any given amino acid, and a practical difficulty in carrying them out is sometimes caused by unsatisfactory readings given by the small amounts present in normal fluids.

The precipitation of protein, determination of nitrogen in the precipitate, and calculation of the material from the results, gives a method which is in some respects undoubtedly better than the one just discussed. The technical error is much smaller than that involved in the amino acid method[3], the nitrogen content of various proteins is apparently more nearly constant than is their amino acid composition, and the method is more closely related to procedures in routine use in most hospital laboratories; to the determination of the non-protein nitrogen in blood, for example.

Although the determination of nitrogen possesses these theoretical advantages over the amino acid determination, certain technical difficulties are found in applying it. These difficulties concern two different parts of the procedure. Small amounts of protein give colors on nesslerization which cannot be easily read, and when large amounts are present the oxidation does not proceed smoothly. An investigation was therefore undertaken to improve the technique in these respects. We have achieved at least partial success in dealing with these problems, and present the method described below in the hope that it will prove useful in other laboratories.

The method is based upon the micro-oxidation and nesslerization previously described for blood proteins[2], but it has been found necessary to modify the technique considerably to meet the difficulties described above. The color obtained upon nesslerization has been increased by making the final determination in a volume of 25 cc. This can be done satisfactorily in the presence of Rochelle salts if less than 0.5 mgm. of nitrogen is present. Oxidation is greatly simplified if the amount of oxidizing reagent is doubled. This lessens the danger of loss of nitrogen from failure to oxidize completely and also reduces the irregularities which result from boiling away of acid. If an aliquot protion is taken for the final determination, it is also possible to remove precipitated phosphates by centrifuging before the color is developed with

Nessler's reagent. Two other factors were also studied. We believe that one of the commonest causes of error in routine laboratories lies in the quantitative transfer of solutions from one container to another, and we have therefore carried out precipitation and oxidation in the same container. We have also felt that further knowledge of the effect of protein precipitants upon the protein of spinal fluid was necessary before a suitable choice of a reagent for routine use could be made, and have therefore carried out some investigations upon this point.

At the present time in this country, tungstic acid and trichloracetic acid seem to be the reagents most generally used for precipitating protein. Since we wished to develop a method which would conform to routine procedures, these reagents were the ones most thoroughly studied, but work was also done with phosphotungstic acid, acetic acid and heat, and acid alcohol. As none of these reagents possessed any advantages over the two first named, they will not be discussed further at this time. It was found in preliminary investigations that tungstic acid possessed some advantages over trichloracetic acid, and that in some ways the latter reagent was to be preferred. Precipitation and recovery was much easier with tungstic acid, but oxidation was quite difficult when large amounts of protein were present, because some of the material reprecipitated in the oxidation mixture and not infrequently escaped oxidation altogether. For a time attempts were made to avoid this source of error, but eventually attention was centered upon improving recovery of the material thrown out of solution by trichloracetic acid. This was finally accomplished by adding methyl alcohol after precipitation. It was found that when this was done amounts of protein as small as that contained in 2 cc. of a 0.005 per cent solution could be easily recovered by centrifuging. This concentration is smaller than is present in normal spinal fluid.

DESCRIPTION OF METHOD

Apparatus: (1) 50 cc. conical centrifuge tubes of pyrex glass, tapering for approximately half their length, and graduated at 10 cc. (2) Adjustable iron clamp to hold centrifuge tubes. (3) Micro burner. (4) Small watch glass to cover centrifuge tubes.

Reagents: (1) 20 per cent trichloracetic acid. (2) Absolute methyl alcohol. (3) Dilute (1:1) oxidizing reagent of Folin and Wu.: (4) 10 per cent Rochelle salts solution. (5) Nessler's reagent prepared according to the directions of Folin and Wu. (6) A solution containing 1.414 grams of pure ammonium sulphate made up to a liter with distilled water. 1 cc. of this solution contains 0.3 mg. of nitrogen. (7) A dilute working standard solution prepared by diluting 10 cc. of the one just described to 100 cc. with distilled water. 1 cc. of this standard contains 0.03 mg. of nitrogen.

Procedure

(*A*) *Precipitation of protein from spinal fluid.* Measure 2 cc. of spinal fluid (a smaller amount of fluid may be taken, and normal salt solution added to give a total volume of 2 cc.) into one of the centrifuge tubes described above. Add 2 cc. of 20 per cent trichloracetic acid. Mix. Heat in a boiling water bath for from 0.5 to one minute. Let cool. Add 6 cc. of absolute methyl alcohol. Mix. Centrifuge. Decant supernatant fluid and drain for 5 minutes on a towel. Washing appears to be unnecessary.

B. Oxidation. Add 2 cc. of the dilute oxidizing reagent of Folin and Wu. Place in the clamp in a slanting position and heat carefully with a low flame of the micro burner until the precipitate has dissolved. Then boil off the water, applying the flame of the burner towards the outer end of the tube. Danger of bumping is diminished if this is done. When the solution begins to turn brown, or when dense white fumes begin to appear, place the tube in an upright position, cover with a watch glass, and continue heating until the contents are colorless. Allow to cool until the fumes have subsided, then tilt the tube and add about 5 cc. of distilled water from a medicine dropper. The water should be allowed to run down the side of the tube, and should be added a drop at a time at first and more rapidly later, if danger from spattering is to be lessened. If a crystalline precipitate forms, it may be broken up with a small glass rod, and the rod rinsed into the tube with a few drops of distilled water. Allow the tube and its contents to cool, and make to 10 cc. with distilled water. Centrifuge if necessary. Transfer with a pipette 5 cc. of the clear supernatant liquid to a second tube graduated at 10 cc. Add 1 cc. of 10 per cent Rochelle salts and make to 10 cc. with distilled water.

C. Preparation of standards. Into a series of tubes graduated at 10 cc. pipette various amounts of the dilute working standard described above. Add to each tube 1 cc. of the dilute oxidizing reagent of Folin and Wu, 1 cc. of a ten per cent Rochelle salts solution, and dilute to 10 cc. with distilled water. For normal spinal fluid the correct amount of nitrogen is usually 0.03 mg., which is contained in 1 cc. of the working standard. It is well, however, to prepare standards containing 0.015 and 0.06 mg. of nitrogen, contained respectively in 0.5 and 2 cc. of the working standard. For spinal fluids containing abnormal amounts of protein stronger standards must, of course, be used. The maximum

is between 0.3 and 0.6 mg., contained respectively in 1 and 2 cc. of the strong standard described.

D. Nesslerization. To each of the standard solutions and the aliquot of the oxidized spinal fluid (each contained in a volume of 10 cc.) add 15 cc. of the Nessler's reagent described by Folin and Wu. Compare in a colorimeter the color of the unknown solution with that of the standard nearest to it in tint, and calculate the result from the formula given below. All solutions should be nesslerized as nearly at the same time as possible, and reading should be made fairly soon after the reagent is added. If the amount of color is slight, and the color of the unknown does not correspond quite closely with that of one of the standards, it is best to read against two standards, and compute the average of the results obtained.

E. Calculation. Calculate the protein content of the spinal fluid from the following formula:

$$\frac{\text{reading of standard}}{\text{reading of unknown}} \times \text{mg. of nitrogen in standard} \times 6.25 \times \frac{100}{1000} = \text{per cent protein}$$

If less than 2 cc. of spinal fluid was taken for the analysis, the result must be multiplied by an appropriate factor. (See foot note).

DISCUSSION

Results of experiments carried out in standardizing the procedure are given in the tables. Table 1 gives results upon blood serum. The total protein content of the serum was first determined by the method of Hubbard and Sly. The serum was then diluted with normal salt solution as shown, and precipitated and nesslerized as described above. The table shows that the method was quite satisfactory when carried out on 2 cc. of a solution containing between 0.2 per cent and 0.005 per cent of protein. Concentrations greater than this could not be oxidized successfully, or nesslerized without prompt precipitation of mercury salts. If protein were present in amounts lower than 0.005 per cent, it could not be satisfactorily centrifuged out of solution, and when nesslerized gave colors so light that they could not be easily read in the colorimeter. Table 1 also shows that purification of the precipitate first obtained by solution in alkali and reprecipitation did not affect the results, and that it seemed to be possible, when analyzing dilute solutions, to use 1 cc. of the oxidizing

solution and nesslerize the entire contents of the tube instead of working with an aliquot of it.

Table 2 contains the results of similar experiments upon spinal fluid. Determination of the protein by difference was impracticable, because the protein contained only a small per cent of the total amount of nitrogen present. It seemed desirable to give some method of estimating the probable accuracy of the determination of various amounts of protein, and the following plan was adopted. The probable protein content of the undiluted spinal fluid was calculated from the results of closely agreeing

TABLE 1

RESULTS ON BLOOD SERUM

PROTEIN IN SERUM	DILUTION	OXIDIZING REAGENT	PRESENT	FOUND	RECOVERED	NOTES
per cent		*cc.*	*per cent*	*per cent*	*per cent*	
3.66	1:50	2	0.0732	0.0720	98.4	Standard method used
	1:50	2	0.0732	0.0735	100.4	Two precipitations
	1:50	1	0.0732	0.0735	100.4	1 cc. oxidizing reagent
	1:100	2	0.0366	0.0364	99.5	Standard method used
	1:200	2	0.0183	0.0183	100.0	Standard method used
	1:200	1	0.0183	0.0193	105.3	1 cc. oxidizing reagent
	1:500	2	0.0073	0.0068	93.0	Standard method used
	1:500	1	0.0073	0.0082	111.5	1 cc. oxidizing reagent
5.75	1:500	1	0.0116	0.0117	100.9	1 cc. oxidizing reagent
	1:1000	1	0.0058	0.0058	100.9	1 cc. oxidizing reagent
6.11	1:25	2	0.244	0.255	104.5	Standard method used
	1:50	2	0.122	0.122	100.0	Standard method used

studies made upon it, and the protein concentrations in the diluted solutions calculated from the figure so determined. The actual results of each experiment upon the diluted material was then compared with this theoretical value, and the difference expressed as per cent. The table shows that when the so-called standard method was followed, that is when 2 cc. of oxidizing reagent were used and an aliquot taken for nesslerization, there was excellent agreement between results upon large amounts and small amounts of the same spinal fluid. The only noteworthy exception to this statement was the results in fluid 1 when 2 cc. of

a fluid containing more than 0.3 per cent of protein was taken for analysis.

Table 3 contains results when 1 cc. of oxidizing reagent was used and the entire specimen treated with Nessler's reagent. Even when small amounts of protein were present these results

TABLE 2

RESULTS UPON DILUTED SPINAL FLUID BY STANDARD METHOD

NUMBER	CALCULATED PROTEIN	DILUTION	PRESENT	FOUND	RECOVERY
	per cent		*per cent*	*per cent*	*per cent*
1	0.335	None	0.335	0.375	112.0
		1:2	0.169(5)	0.167	99.6
		1:4	0.0838	0.0840	99.1
2	0.163	None	0.163(5)	0.163	99.6
		1:2	0.0808	0.810	99.6
		1:4	0.0409	0.0410	100.2
3	0.149	None	0.149	0.149	100.0
		1:2	0.0745	0.0745	100.0
		1:4	0.0378	0.0378	100.0
4	0.217	1:2	0.108(5)	0.110	101.4
		1:4	0.0542	0.0535	98.7
5	0.0665	None	0.0665	0.0690	103.8
		1:4	0.0166	0.0160	96.4
6	0.0537	None	0.0537	0.0520	96.8
		1:2	0.0269	0.0275	102.2
7	0.101	1:2	0.0505	0.0513	101.5
		1:4	0.0253	0.0250	98.8
8	0.0500	None	0.0500	0.0509	100.8
		1:2	0.0250	0.0250	100.0
		1:4	0.0125	0.0125	100.0
9	0.0404	1:2	0.0202	0.0203	100.5
		1:4	0.0101	0.0101	100.0
10	0.0184	None	0.0184	0.0183	99.3
		1:2	0.0092	0.0093	100.6

did not agree as closely as did those shown in table 2, and this method, therefore, does not seem to be as satisfactory for routine use as is that involving the use of 2 cc. of oxidizing reagent and the nesslerization of an aliquot of the solution.

Table 4 contains results of other procedures used in controlling the technique proposed. The table shows: (1) That when the

precipitate first obtained was redissolved in alkali and reprecipitated with trichloracetic acid and methyl alcohol, the same result was obtained as when the standard procedure using only one precipitation was employed. Purification of the precipitate seems, therefore, to be unnecessary. (2) That when fairly large amounts of protein were present results were identical when trichloracetic acid was used alone and when it was combined with methyl alcohol. Since very small amounts of precipitate cannot be centrifuged out of solution in the absence of methyl alcohol, extension of these experiments to the study of normal spinal fluids

TABLE 3
RESULTS OBTAINED WHEN 1 CC. OF OXIDIZING REAGENT WAS USED

NUMBER	CALCULATED PROTEIN	DILUTION	OXIDIZING REAGENT	PRESENT	FOUND	RECOVERY
	per cent		*cc.*	*per cent*	*per cent*	*per cent*
11	0.068	None	1	0.068	0.068	100.0
		1:2	1	0.034	0.034	100.0
		1:4	1	0.017	0.015	89.6
12	0.0406	1:2	2	0.0203	0.0203	100.0
		1:4	2	0.0102	0.0101	99.5
		1:4	1	0.0102	0.0102	100.5
13	0.0101	None	2	0.0101	0.0100	99.5
		None	1	0.0101	0.0113	112.0
		1:2	1	0.0050	0.0051	100.2
14	0.059	1:2	1	0.0295	0.0375	127.0
		1:4	1	0.0148	0.0113	77.8

is impracticable. (3) That when small amounts of protein were present precipitation with tungstic acid and with trichloracetic acid gave results which were almost identical, but that when the concentration was high the agreement was not good. It seems probable that loss during oxidation, which has been discussed already, explains the discrepancies. (4) That when phosphotungstic acid was used in studying normal spinal fluid the result was much higher than that obtained with trichloracetic acid and methyl alcohol. It seems probable that non-protein nitrogen compounds precipitated by phosphotungstic acid are sometimes present in spinal fluid and make this reagent an unsuitable one for studying the protein content of this material.

Table 5 shows the distribution of thirty-one values obtained by the method described, upon various samples of fluid submitted to

TABLE 4
STUDIES OF METHODS OF PRECIPITATING PROTEIN FROM SPINAL FLUID

NUMBER	DILUTION	OXIDIZING REAGENT	STANDARD METHOD	MODIFIED METHOD	DESCRIPTION OF METHOD
		cc.	*per cent*	*per cent*	
15	1:2	2	0.104	0.101	No methyl alcohol used
16	1:2	2	0.0745	0.0755	No methyl alcohol used
17	None	2	0.0280	0.0272	Precipitated twice with trichloroacetic acid
18	None	1	0.0316	0.0291	Precipitated twice with trichloroacetic acid
19	1:2	2	0.110	0.102	Precipitated twice with tungstic acid
20	None	2	0.163	0.156	Precipitated twice with tungstic acid
21	None	2	0.193	0.171	Precipitated twice with tungstic acid
22	None	2	0.100	0.107	Precipitated twice with tungstic acid
23	None	2	0.0536	0.0536	Precipitated twice with tungstic acid
24	1:2	2	0.0520	0.0495	Precipitated twice with tungstic acid
25	1:2	2	0.0192	0.0183	Precipitated twice with tungstic acid
26	1:2	2	0.0520	0.0510	Precipitated twice with phosphotungstic acid
27	None	2	0.0163	0.0183	Precipitated twice with phosphotungstic acid

TABLE 5
DISTRIBUTION OF RESULTS ON CEREBROSPINAL FLUID

PROTEIN	NUMBER SPINAL FLUIDS	PROTEIN	NUMBER SPINAL FLUIDS
per cent		*per cent*	
0.005 to 0.014	1	0.075 to 0.084	1
0.015 to 0.024	8	0.085 to 0.094	0
0.025 to 0.034	3	0.095 to 0.104	1
0.035 to 0.044	1	0.105 to 0.204	7
0.045 to 0.054	4	0.205 to 0.304	1
0.055 to 0.064	1	Over 0.304	2
0.065 to 0.074	3		

the laboratory for analysis. The table shows that only a small number of the fluids, three, or 10 per cent of the total, contained

more protein than could be determined when 2 cc. was taken for analysis. The normal value lies between 0.02 and 0.03 per cent by this method.

CONCLUSION

A convenient method for determining the protein in spinal fluid is described. The method depends upon the use of trichloracetic acid and methyl alcohol as precipitating reagents, and the determination of nitrogen by a modified Folin-Wu technique. It is applicable to 2 cc. samples of spinal fluid containing approximately from 0.005 to 0.25 per cent of protein, and can be used in studying any higher concentration by diluting smaller amounts of fluid before analyzing them. The lowest protein concentration found was approximately 0.015 per cent, and the normal value appeared to lie between 0.02 and 0.03 per cent.*

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* It is possible to use the following variation of the method described when only small amounts of protein are present: Add 1 cc. of oxidizing mixture instead of 2 cc. Oxidize as described. Add 1 cc. of 10 per cent Rochelle salts, and make to a volume of 10 cc. Add 15 cc. of the Nessler's reagent of Folin and Wu to the entire contents of the tube. Remove any precipitate which may be present by centrifuging. Read and calculate as described, but divide the final result by two. This method gives more color after nesslerizing than does that described in the text, but the results obtained by it do not appear to be as uniform.

THE SENSITIVITY OF WASSERMANN ANTIGENS IN RELATION TO RATE OF DILUTION*

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There is evidently some reason why different laboratory workers have such a varied preference for some one of a large assortment of antigens used in the Wassermann test. The antigen is the most important factor in the test, and quantitatively and qualitatively the most varied. Serologists have reported widely different results in the past but these differences are becoming less noticeable because of more general agreement in the use of the more desirable types of antigens.

Neymann and Gager[2] have worked out physical separation of the various ether, acetone, and alcohol soluble constituents of beef heart after the preliminary extraction with ether. Then after extracting this ether extracted heart with absolute ethyl alcohol, they found the acetone insoluble lipoid of this alcoholic extract, or diaminophosphatid, to be the most antigenic substance in the heart muscle. They recommended the use of this alcoholic extract containing 0.2 per cent cholesterol. They did not state the exact proportion the alcohol should bear to the ether extracted heart powder, but stated that the alcohol should cover the powder to a depth of half an inch and also to make a similar second extract, which might be better than the first.

This indefinite proportion of alcohol to the heart muscle powder was given probably because they were particularly interested in the diaminophosphatid or acetone insoluble fraction after the alcohol was evaporated. By the method of testing given later in this paper there were found only slight differences in antigens made in the wide range of from 6 to 14 cc. of alcohol used in the

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extraction of each gram of ether-extracted heart powder. So the indefiniteness of the recommendations is almost justified except on the grounds of economy. I have confirmed their statement that a second alcoholic extract is very slightly stronger than the first. A third extract is very slightly stronger still than the second, if the total alcohol used for the three extractions does not exceed 14 parts.

The ether extractions were made in a Soxhlet apparatus, the usual time for extraction being eight hours, but it seemed to make no difference when the extraction was continued for eighteen hours or more.

This antigen is mentioned in some detail because it is not complex, is easily prepared, different batches vary hardly at all, and because it has been used routinely for over sixteen years, and in connection with these experiments. In recent years only Difco powdered beef heart has been used, instead of freshly dried hearts.

It is well known that different antigens require different methods of dilution to make them more sensitive. There are two common basic methods of dilution. The less common method of diluting antigen by rubbing the dry lipoid with diluent will not be discussed.

Rapid dilution is defined as the adding of antigen drop by drop to the diluent, preferably with agitation of the mixture during the addition, or the squirting of the antigen into the diluent, or any method in which the antigen is added to the total bulk of the diluent and mixed at the same time, preferably with violent agitation. The technic of adding antigen drop by drop to salt solution may be slow, but each drop added is in reality a rapid dilution of a small quantity of antigen. All these rapidly diluted mixtures seem identical in appearance and properties. Slow dilution is the adding drop by drop of the diluent to the antigen, with agitation after each addition, covering in all a period of about five minutes (or longer) for the total mixture. It must be distinctly understood that adding salt solution slowly drop by drop to antigen is practically the direct opposite to the adding of antigen slowly drop by drop to the salt solution, this latter method being similar in character to the rapid mixing of the antigen and salt solution.

It is well known that plain antigens usually are most antigenic when they are diluted slowly. It does not seem so well known that highly cholesterolized antigens work best when diluted rapidly. Apparently it is the proportion of diaminophosphatid or its substitute, lecithin, to the cholesterol (or other sterol) which determines the preferable method of mixing. If the amount of diaminophosphatid is double that of the cholesterol, the antigen is more antigenic if diluted slowly. If the amount of cholesterol is double that of the diaminophosphatid, the antigen is more antigenic if diluted rapidly. As a solution of pure diaminophosphatid has added to it increasing amounts of cholesterol, the differences in antigenic values between the slowly diluted and rapidly diluted antigens become less and less until a point is reached where neither method seems preferable. But beyond that point, the higher the cholesterol ratio the more important is it that the antigen should be diluted rapidly.

Kolmer[1] in a recent paper recommended adding his antigen slowly to the diluent. He also gave references which show that some antigens with a relatively low cholesterol content seem to work best when diluted rapidly, and that some antigens with an appparently high cholesterol content work best when diluted slowly. Further work will be necessary to find out the cause of the apparent contradictions.

It may be that the best in antigens cannot be attained by assuming that rapid dilution and slow dilution are the only basic methods. Various combinations of these methods may be of importance. Other substances in antigens may markedly change the effect of the quantitative relationship between active lipoid and sterol.

It is the usual custom to test antigenic activity by using a fixed dose of some fairly strongly positive serum and then in the tests to use a certain number of so-called antigenic units based on the activity of this positive serum of unknown strength. Probably a better method is to use as much antigen as possible without getting any anticomplementary reactions. There are a few workers who do not use a strongly positive control serum in their routine tests, but rather a weakly positive serum. A weakly positive serum is preferable for testing antigens.

Ottenberg[3] said

The dose of antigen which gives fixation with the highest dilution of positive serum is the optimal dose, and this is not by any means the largest dose tested.

This observation would suggest that he had noted the occurrence of an antigen zone above which and below which the antigen doses would show decreased sensitivity. But he further explained

The probable explanation of this observation is that when the dose of antigen is increased beyond a certain point, the amount of complement which has to be added to overcome the anticomplementary effect is too great to be fixed by certain grades of positive serum.

If the other systems (Wassermann, Citron, Noguchi, Walker and Swift) in which the dose of complement is either fixed (0.1 cc.) or is two hemolytic units, the rule does not apply because the ratio of complement to antigen is always greater and hence the more one can increase the antigen dose (and still remain below the point where non-specific fixations are obtained) the better the results.

These zones of optimal sensitivity have been well known to workers in contagious abortion for over twenty yeats. But, these zones are less well known in the Wassermann reaction, probably because most of the antigens have been so anticomplementary that the zone could hardly be detected. These zones of maximum sensitivity do not require any special Wassermann technic to bring them out. Necessary conditions are the use of a weakly positive serum, a close hemolytic titration to avoid turning the weak positive fixation into a negative, and an antigen which is very sensitive and very free from anticomplementary properties.

METHOD OF TESTING ANTIGEN

The quantities of reagents used usually have been 0.1 cc. or 0.2 cc. of human serum, a series of doses of antigen, 0.5 cc. of 5 per cent guinea pig serum for complement, just enough antisheep amboceptor for complete hemolysis in one-half hour, and 0.2 cc. of a 6.25 per cent suspension of sheep corpuscles.

The antigen and human serum are mixed together and allowed to stand for ten minutes or more before adding the complement, not with any view to increasing activity, but for an opposite or protective effect of normal human serum against anticomplementary action of antigen. Normal human serum, when previously mixed with antigen and allowed to stand ten minutes or more, protects guinea pig complement against anticomplementary action of antigen

to the extent of permitting practically complete hemolysis, even when the antigen control of the same dosage of antigen shows just complete inhibition of hemolysis.

With any particular antigen, the method of testing is to take a weakly positive serum and, keeping other reagents constant, to vary the dosage of antigen to bring out the optimal dose, above which and below which the reaction is weaker or negative. If a weakly positive serum is not available, it may be necessary to use stronger serum in two or three dilutions to detect this optimal zone. It is better to dilute the stronger serum with negative serum to make it equivalent to a weakly positive serum. It is best to make two series, one with a weakly positive serum, and one with another serum which just completely inhibits hemolysis with the most sensitive antigen routinely used. This variation of serum will prevent disappointment which might follow the all too common variation of the hemolytic system in the direction of increased hemolysis. These titrations must be carried out in duplicate, the one with a slow method and the other with a rapid method of diluting antigen.

SUMMARY

Antigens should be tested for optimal dosage with a weakly positive serum, and in slow and in rapid dilutions for optimal sensitivity and optimal dosage. Relatively high lipoid content suggests slow dilution, and relatively high sterol content suggests rapid dilution as being preferable.

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EDITORIAL

Vaccination Against Acute Anterior Poliomyelitis

During the past year interest has been greatly renewed in the possibility of safely and effectively vaccinating human beings against acute anterior poliomyelitis. Since about 90 per cent of cases occur in children it would appear highly desirable to protect them against this dreaded disease until the natural immunity of adult age has been attained.

Fortunately the virus is transmissible to monkeys and the possibility of successfully vaccinating these animals against it has engaged the interests of numerous investigators here and abroad since the initial attempts of Flexner and Lewis[1] in 1910, who found that subcutaneous and intracutaneous injections of living virus sometimes resulted in active immunization. The method, however, was considered too dangerous for human beings and since then other investigators have employed virus killed or inactivated by heat and chemical agents or mixtures of virus and immune serum.

The general results of these investigations indicated, however, that killed virus was probably of low immunizing value and that successful vaccination appeared to demand the administration of living virus, as has been found true in some other virus diseases of man and of the lower animals.

During the past year, almost simultaneous reports of the successful vaccination of monkeys with two entirely different vaccines were made by Dr. William H. Park and Dr. Maurice Brodie of New York and by Dr. John A. Kolmer of Philadelphia the former using virus apparently killed with formalin and the latter a living virus slightly attenuated with sodium ricinoleate. During 1933 Kolmer and Rule[2] had found that vaccines inactivated by heat and chloroform were without immunizing value in the doses employed while that attenuated with sodium ricinoleate

effectively and safely vaccinated some monkeys as had been previously observed in a few animals by McKinley and Larson[5]. Subsequently Kolmer and Rule[3] reported that a modification of the vaccine carrying 4 per cent monkey spinal cord virus in a 1 per cent solution of sodium ricinoleate safely and effectively immunized a group of eighteen monkeys.

While the details of the investigations of Park and Brodie have not been published, their vaccine was first administered to a group of volunteers and later to children. At the same time Kolmer and Rule first took their vaccine themselves with no ill effects and with the production of antiviral antibody and then immunized a group of twenty-five children varying in age from 8 months to 15 years, reporting no ill effects and the production of antibody in about 85 per cent of the group.[4]

The duration of the immunity cannot be stated at present except to mention that according to Dr. Park antibody may disappear from the blood of children immunized with the formalin killed vaccine in about 5 months while Kolmer has reported that monkeys vaccinated with his vaccine have been found immune to intracerebral inoculations of virus as long as 3 years later. But if the immunity only protects children over their age of greatest susceptibility it would be well worth while.

It appears that large doses of the formalin killed vaccine of Park and Brodie are required as single amounts as large as 5 cc. are given to children while the attenuated vaccine of Kolmer is given in three small subcutaneous injections at weekly intervals for a total of about 1.5 to 2.0 cc. From the results so far available it would seem therefore that formalin killed virus may produce immunity but that the amount required is much larger than in the case of the sodium ricinoleate antigen and that the immunity is of shorter duration.

Naturally the matter of safety in giving subcutaneous injections of living virus is one of paramount importance even though apparently attenuated to some slight degree by sodium ricinoleate. But as Kolmer has pointed out it is highly probable that the remote monkey passage virus used in the preparation of his vaccine has lost its infectivity for human beings and especially since it is

administered by subcutaneous injection in relatively small doses. At least absolutely no ill effects have been observed in monkeys receiving very much larger does per kilogram of weight and it is reported that over 6000 children have been vaccinated with no ill effects whatsoever aside from mild local reactions at the sites of injection.

Furthermore it would appear that the attenuated vaccine is apparently capable of producing immunity very rapidly since antibody has been found in the blood of three children and in monkeys as early as 4 days after the first dose (5). Additional evidence of this early antibody production is reported in this issue of the JOURNAL; some monkeys vaccinated as late as 5 days after intracerebral inoculations of virus have remained perfectly well. This prompt antibody production may be an additional factor of safety aside from indicating that the vaccine may be safely and effectively given during epidemics of the disease.

It would appear therefore that two vaccines are now available for vaccination against acute anterior poliomyelitis although only further investigation can reveal the relative merits and effectiveness of each. In this connection it may be stated that each is prepared from remote monkey passage virus and it remains to be determined if the antibody produced will effectively protect against what may be called "human virus" responsible for both isolated and epidemic cases of the disease, especially since Paul and Trask[6] have shown slight differences in the immunological aspects of different strains of virus. In this connection it may be stated, however, that Kolmer and Rule have found that antibody produced in children by their attenuated vaccine was found capable of neutralizing human virus in the third monkey transfer from the recent California epidemic.

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NEWS AND NOTICES

On June 6th LaSalle College conferred the honorary degree of Doctor of Science, and on June 11th, St. Joseph's College conferred the honorary degree of Doctor of Humane Letters in recognition and appreciation of Dr. Kolmer's achievements and contributions to medical science with special reference to his work on immunization against acute anterior poliomyelitis. Dr. Kolmer is Professor of Medicine at Temple University along with Dr. Chas. L. Brown of the University of Michigan, the chair being divided to permit Dr. Kolmer to devote more of his time to research work.

It will be of considerable interest to members of the Society to examine certain provisions of the Social Security Act recently passed by the Congress. Not only does it provide certain funds for mothers and children but sets aside a fund of $8,000,000 to be distributed to the Public Health Departments of states and $2,000,000 as a research fund for the United States Department of Public Health.

The Food and Drug Act, so important from a medical standpoint, was not acted upon.

The preparation of the second edition of Approved Laboratory Technic has gotten under way. It is now expected to get the manuscript of the book into the hands of the printers by January first so the new edition will be out in June, 1936.

A conference has been called by Secretary Wallace to consider the future of Biological Abstracts. Funds, usually available for its publication, have not been re-appropriated and unless aid is immediately procured, this most important publication will be unable to continue. It is hoped the conference will be able to solve the problem.

A new fifth edition of Difco Manual of Dehydrated Culture Media and Reagents has been issued. It contains necessary and valuable information. Members of the Society can secure copies on request of the Difco Laboratories whose advertisement will be found in this JOURNAL.